Autonomic Nervous System Activity and Menopausal Symptoms

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THESIS

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LIST OF ABBREVIATIONS

ANS	Autonomic Nervous System
AUC	Area under the Curve
BAI	Beck Anxiety Inventory
BMI	Body Mass Index
BOLD	Blood Oxygen Level Dependent
CAMS	Cognition and Menopausal Symptoms
CES-D	Center for Epidemiological Studies, Depression Scale
CCTS	Center for Clinical and Translational Science
fMRI	Functional Magnetic Resonance Imagining
FSH	Follicular Stimulating Hormone
GCS	Greene Climacteric Scale
HF	Hot Flash
HFs	Hot Flashes
HPG	Gypothalamic-Pituitary-Gonadal Axis
HRV	Heart Rate Variability
LH	Luteinizing Hormone
MFQ	Memory Functioning Questionanire
MPA	Medroxyprogesterone Acetate
MRM	Mixed-effects Regression
PSG	Polysomnography
PSQI	Pittsburgh Sleep Quality Index

LIST OF ABBREVIATIONS (continued)

PSS	Perceived Stress Scale
REM	Rapid Eye Movement
RSA	Respiratory Sinus Arrhythmia
sAA	Salivary Alpha-amylase
SMWHS	Seattle Midlife Women's Health Study
STRAW	The Stages of Reproductive Aging Workshop
SWAN	The Study of Women's Health Across the Nation

SUMMARY

Hot Flashes (HFs) occur in up to 75% of women transitioning through menopause. Previous research suggests objective HFs may shift the balance between the calming branch (parasympathetic) and the 'fight or flight' branch (sympathetic) of the autonomic nervous system (ANS) through withdrawal of calming activity. There is limited evidence about the effect of HFs on the 'fight or flight' branch of the ANS. Independent of ANS changes, HFs are associated with decreased cardiovascular health (CVH). The link between CVH and the physiology of HFs is not understood. Here we investigate differences in ANS function between women with frequent or infrequent self-reported and objectively detected HFs.

Participants included 40 midlife women (Mean age = 52.1) from a parent study investigating associations between menopausal symptoms and cognition: half reported frequent HFs (>30/ week) and half reported infrequent HFs (<7/ week). HFs were assessed with objective monitoring and subjective reporting. Parasympathetic activity was assessed with variability in heart rate (HRV), and sympathetic activity was measured using salivary alpha-amylase (sAA). Results: HRV changed across body position ($p<0.05$) but not between self-reported or objective HF group ($p's>0.40$). At thirty minutes after wake, women with objective HFs had an attenuated awakening response of sAA compared to women without objective HFs ($\beta=0.71$, SE= 0.32, $p=0.03$, $d=0.73$).

Our findings support a state shift in autonomic balance towards increased 'fight or flight' activation with objective, but not self-reported, HFs. Understanding the link between ANS activity and the physiology of HFs may give insight for reducing cardiovascular disease risk and treating HFs.

I. INTRODUCTION

The primary aim of this project is to investigate potential differences in autonomic nervous system (ANS) function (sympathetic and parasympathetic) between women who are experiencing subjective and objective hot flashes (HFs). A secondary aim of this study is to investigate if psychological outcomes such as memory functioning and sleep quality are related to any observed differences in ANS function.

There is limited evidence about the effect of HFs on the 'fight or flight' branch of the ANS. Previous research suggests objective HFs may shift the balance between the calming branch (parasympathetic) and the 'fight or flight' branch (sympathetic) of the ANS through withdrawal of calming activity, causing heightened activity within the sympathetic branch (Matsukawa, Sugiyama, Watanabe, Kobayashi, & Mano, 1998; Narkiewicz et al., 2005; Simonian, Delaleu, Caraty, & Herbison, 1998). HFs have been linked with transient increases in one of the main neurotransmitters of the ANS, norepinephrine (Cignarelli et al., 1989; Freedman, 1998; Kronenberg, Cote, Linkie, Dyrenfurth, & Downey, 1984; Kronenberg & Downey, 1987). The shift in the balance between sympathetic and parasympathetic drive may be contributing to an increased risk for cardiovascular disease and may also be the link between vasomotor symptoms and cognitive dysfunction and other psychological outcomes in menopausal women. Compared to subjective HFs, objective HFs show a stronger relationship with physiological and cognitive outcomes. Specifically, only objective HFs, but not subjective HFs, are associated with altered heart rate variability (HRV) and memory dysfunction in midlife women (Maki et al., 2008; Freedman et al., 2011; Thurston et al., 2010, 2012). The link between increased risk for cardiovascular disease and cognitive dysfunction and the physiology of HFs is not well

understood. Here we investigate differences in ANS function between women with frequent or infrequent self-reported and objectively detected HFs.

This investigation aims to compare markers of ANS function in women with infrequent versus frequent HFs; specifically, utilizing HRV as a marker for parasympathetic nervous system activity and salivary alpha-amylase (sAA) as a marker of sympathetic nervous system activity. HRV has been used within research as a marker of parasympathetic activity; however, it is also associated with clinical outcomes of cardiovascular disease and mortality (Bigger et al., 1992; Hillebrand et al., 2013; La Rovere, Bigger, Marcus, Mortara, & Schwartz, 1998; Wolf, Varigos, Hunt, & Sloman, 1978). The use of sAA as a marker of sympathetic activity is a developing research technique, but sympathetic nervous system activity is integral in the understanding of the etiology of HFs (Freedman, Kruger, & Wasson, 2011; Thurston, Christie, & Matthews, 2010, 2012) and their potential effects on health and well-being. Additional exploratory analyses focused on the extent to which vasomotor-associated changes in ANS activity account for differences in psychological outcomes between these groups of midlife women.

II. SCIENTIFIC BACKGROUND

HFs occur in up to 75% of women transitioning through menopause (Kronenberg, 1990). HFs decrease quality of life (Woods & Mitchell, 2011), and are associated with negative health outcomes such as poorer cardiovascular health, (Ozkaya et al., 2011; Thurston et al., 2011), depressive and anxiety symptoms (Freeman, Sammel, & Lin, 2009), and sleep disturbances (Brown, Gallicchio, Flaws, & Tracy, 2009; Kravitz et al., 2008; Sowers et al., 2008). We recently reported that frequent physiological HFs, or HFs detected using an objective monitor, predict poorer memory in menopausal women, but subjective HFs did not. Specifically, physiological HFs during sleeping hours was the best predictor of memory dysfunction. These findings point to the utility of objective measurement of HFs when understanding the relationship between HFs and other symptoms. I extended this area of inquiry to understand the relationship between HFs and ANS function in women transitioning through the menopause.

It has long been thought that the ANS is involved in vasomotor symptom physiology (Ginsburg, Swinhoe, & O'Reilly, 1981). Women with vasomotor symptoms show evidence of an imbalance between sympathetic and parasympathetic nervous system activity. Specifically, symptomatic midlife women have elevated basal blood pressure, suggestive of increased sympathetic drive (Gallicchio, Miller, Zacur, & Flaws, 2010; Tuomikoski, Haapalahti, Sarna, Ylikorkala, & Mikkola, 2010; Tuomikoski, Haapalahti, Ylikorkala, & Mikkola, 2010); however, each HF is associated with a transient decrease in heart rate and blood pressure (Germaine & Freedman, 1984). Measures of HRV also suggest an imbalance between the sympathetic and parasympathetic nervous system during a HF (Ford, Slade, & Butler, 2004; Freedman et al., 2011; Thurston et al., 2010, 2012). Finally, there are transient increases in serum epinephrine and

decreases in serum norepinephrine following a HF (Cignarelli et al., 1989; Kronenberg et al., 1984; Swartzman, Edelberg, & Kemmann, 1990).

i. Menopause

The menopausal transition is characterized by the gradual cessation of ovarian functioning. Currently in the United States the average age of menopause is 51 (te Velde & Pearson, 2002). Decline in ovarian function results in an erratic pattern of circulating sex steroid hormones, including estrogen and progesterone, during the perimenopausal stage and consistently low levels during the postmenopausal stage. During the reproductive years in a woman's life, the menstrual cycle is characterized by cyclical changes in hormones which control ovulation. The menstrual cycle can be divided into three distinct phases: the follicular phase, ovulation and the luteal phase. The follicular phase begins on the first day of menstruation and is characterized by relatively low levels of both estrogen and progesterone. During the late part of the follicular phase, levels of follicular stimulating hormone (FSH) rise and cause maturation of follicles within the ovary. These follicles begin to produce increasing amounts of estradiol which eventually results in a surge of luteinizing hormone (LH). This surge in LH causes the most developed follicle to mature into an oocyte. During ovulation, levels of both FSH and LH drop as the corpus luteum begins to produce increasing levels of progesterone and moderate levels of estradiol. During the luteal phase, if fertilization of the oocyte does not occur, levels of FSH and LH will drop below what is required to maintain the corpus luteum. This disintegration will cause levels of progesterone and estrogen to decline, prompting the onset of menstruation.

During the menopausal transition, the disruption of this normal feedback loop within the hypothalamic-pituitary-gonadal (HPG) axis causes variable levels of both FSH and estrogen (Burger, 1994). During the transition the number of follicles in reserve within the ovary

dwindles. This decrease in follicles disrupts the normal feedback cycle which normally decreases FSH levels. The result is high levels of FSH in women as they become postmenopausal, as increasing levels of FSH are required to cause the pre-ovulatory surge in estrogen levels from the remaining follicles.

The Stages of Reproductive Aging Workshop (STRAW) categorized the criteria for staging reproductive aging (Soules et al., 2001). The continued development of these categories is based upon research on the cessation of the menstrual cycle, fertility, and the associated changes in the hypothalamus, ovaries, and consequent bleeding patterns. Both workshops concluded that patterns of menstruation are the best predictors of menopausal status (Harlow et al., 2012; Soules et al., 2001). The most recent workshop has segmented the menopausal transition into three broad categories: reproductive, menopausal transition, and postmenopause (Harlow et al., 2012). The reproductive stage is the time between menarche through midlife when ovarian function begins to decline. This stage is characterized by women having regular menstrual cycles. In the late reproductive stage there are declines in fertility accompanied by changes in menstrual flow. Specifically, menstrual flow can become heavier, or lighter and last for more or less time when compared to menstrual flow during the reproductive stage. The menopausal transition includes three subcategories: early perimenopause, late perimenopause and the first year of postmenopause. Early perimenopause is defined as a persistent change in cycle length by at least seven days, across two or more consecutive cycles. Late perimenopause is defined by amenorrhea, or lack of menstruation, for at least sixty days. Amenorrhea of twelve consecutive months is the transition point into the early postmenopausal stage. The distinction of being post-menopausal can only be made after the transition has passed; thus it is included within the menopausal transition category. Early postmenopause includes the first six years following the

final menstrual period, with late postmenopause including the rest of the lifespan (Harlow et al., 2012).

The menopausal transition manifests in different symptoms, which are often attributed to the decline in ovarian hormones. In the Study of Women's Health Across the Nation (SWAN), the most common menopausal symptoms were vasomotor in nature, including HFs and night sweats (Gold et al., 2000; Kronenberg, 1990). Other common menopausal complaints of women include changes in cognitive functioning (Woods, Mitchell, & Adams, 2000), sleep disturbances (Baker, Simpson, & Dawson, 1997; Brown et al., 2009; Kravitz et al., 2008; Sowers et al., 2008), and mood changes (Freeman et al., 2009; Freeman et al., 2005).

ii. HFs

HFs are the most frequently reported and recognizable symptom of the menopausal transition (Gold et al., 2000). As many as 75% of women experience HFs during the menopause transition, and 15% of symptomatic women report severe HFs (Gold et al., 2000; Kronenberg, 1990). HFs are described as intense sensations of heat though the neck, chest, and face often accompanied by sweating. Vasomotor symptoms include both HFs and night sweats, intense sweating episodes that occur during sleep. In addition to physical sensations, HFs are also associated with psychological symptoms, such as feelings of anxiety and distress (Freedman, 2000). The physical and mental symptoms can range in frequency, intensity and duration (Kronenberg, 1994). HFs have been associated with decreased quality of life (Woods & Mitchell, 2011), and are linked with negative health outcomes such as poorer cardiovascular health, (Ozkaya et al., 2011; Thurston et al., 2011), increased depressive and anxiety symptoms (Freeman et al., 2009), and sleep disturbances (Brown et al., 2009; Erlik et al., 1981; Kravitz et al., 2008; Sowers et al., 2008).

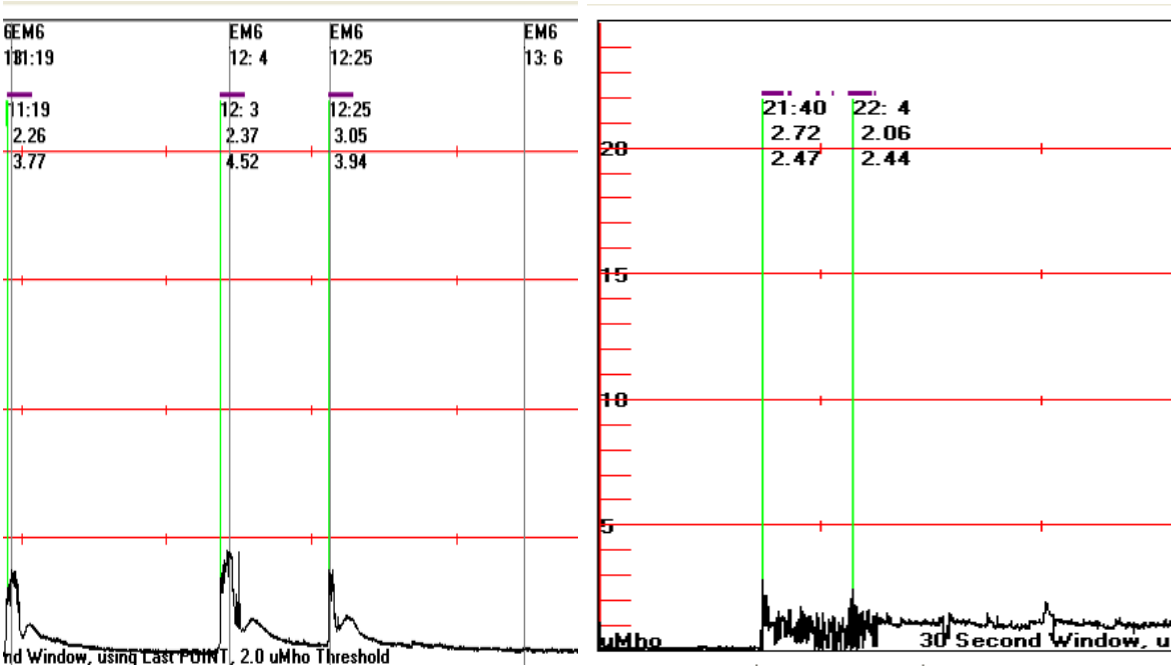
The frequency of HFs peaks during the perimenopause into the early postmenopausal period; however, some women may experience HFs into their eighties (Kronenberg, 1990). Women also under-report the number of HFs they are having by as much as 60% (Maki et al., 2008a). A recent report from the Seattle Midlife Women's Health Study has linked subjective HFs to reported workplace interference (Woods & Mitchell, 2011). This may be particularly relevant due to increasing career and personal life demands that are concurrent with the peak of vasomotor symptoms (Woods & Mitchell, 2011). In addition to changes in physical health, perception of health is one of the largest predictors of workplace interference in midlife women (Woods & Mitchell, 2011).

The Food and Drug Administration currently considers self-reported HFs the gold standard for clinical trials; however, recent advances in vasomotor symptom research have developed an objective way to measure HFs using ambulatory skin conductance monitors. These monitors have high consistency within monitoring sessions, show few false negatives and only detect HFs in symptomatic women (Carpenter, Andrykowski, Freedman, & Munn, 1999; Freedman, Norton, Woodward, & Cornelissen, 1995). Moreover, they show high correlation with subjective HFs in laboratory settings. Sternal skin conductance HF monitors are highly reliable with up to 95% sensitivity in a laboratory setting (Freedman, 1989). In ambulatory settings, women detect between 57% and 64% of daytime physiological HFs, and 22% to 50% of nighttime HFs (Carpenter, Monahan, & Azzouz, 2004; Maki et al., 2008a). Objective HF monitors record skin conductance on the sternum of symptomatic women (Freedman, 1989). Skin conductance during HFs has a very distinct waveform that can be easily distinguished from sweating due to heat or activity (Figure 1). The standard waveform for a HF is a rapid rise in skin conductance followed by a gradual taper. Conversely, waveforms caused by sweating from

heat or exertion have very rapid transitions between high and low levels of skin conductance, which appear as a jagged line. This distinction in the waveform between HFs and sweating through visual inspection allows for more precision in identifying HFs from unrelated sweating. While large epidemiological studies have not yet adopted this technology, their findings have directed the research looking into mechanisms by which HFs can affect quality of life and general health. Research utilizing the objective HF monitors may have higher sensitivity at detecting events which are correlated with physiological changes, such as changes in mental abilities (Maki et al., 2008a) or cardiovascular health (Freedman et al., 2011; Thurston et al., 2010, 2012).

Figure 1. Graphical Output from the Biolog Hot Flash Monitor Showing the Distinction between Representative Hot Flashes in the Left Panel and Sweating in the Right Panel.

Hot Flashes Sweating



Note: The green lines represent detection of the 2.0 mmho threshold by the computer software. Black lines topped with "EM6" are even markers where the subject demarcated that they were experiencing a hot flash.

1. The Neurophysiology of HFs

The hypothalamus, and specifically the preoptic area, are the main brain regions tasked with regulating body temperature (Dimicco & Zaretsky, 2007). Humans are homeothermic, meaning they regulate body temperature at a fairly constant level through both changes in metabolism and behavioral modification (Dimicco & Zaretsky, 2007; Romanovsky, 2007). The control of heat dissipation is mainly modulated by warm-sensitive receptors in the medial preoptic area. These receptors signal that vasodilation and sweating are needed to cool the body. Subsequently, the signal is transduced through the ventral tegmental area and periaqueductal grey and the peripheral vascular response is modulated though cholinergic projections via the sympathetic nervous system, specifically the stellate ganglion (Nagashima, Nakai, Tanaka, & Kanosue, 2000). Acetylcholine acts at the level of sweat glands to rapidly cool the body (Kazuyuki, Hosono, Zhang, & Chen, 1998).

Perception of temperature is regulated mainly through the insular cortex. Classically, the insula is cited as a sensory perception area of the brain. It also has a large number of inputs from the thalamus (Nieuwenhuys, 2012). Recent neuroimaging data has suggested the insula may play a role in detection and awareness of thermoregulatory events, including HFs. Bilateral insula fMRI activity was negatively correlated with subjective temperature ratings to increasingly cold stimuli in a group of ten adults (Craig, Chen, Bandy & Reiman, 2000). Measurement of galvanic skin conductance, a proxy of sympathetic nervous system arousal, in healthy adults has also been linked with increased insular cortex activity during a decision making task. During a HF, women have increases in blood oxygen level dependent (BOLD) signal in the insula (Freedman, Benton, Genik, & Graydon, 2006). Furthermore, baseline metabolism in the insula and hypothalamus has been shown to be useful in predicting which

women may develop HFs. Specifically, baseline levels of glucose metabolism in the hypothalamus and insula were lower in women who developed HFs after pharmacological suppression of ovarian hormones with leuprolide acetate therapy (Joffe et al., 2012). These data suggest that the insula activity may differ in women who do and do not develop HFs as they transition through menopause.

Many hormones and neurotransmitters have been implicated in the physiology of HFs. The onset of HFs occur at times of estrogen withdrawal, caused by natural or surgical menopause and pharmacological suppression of HPG axis function; however HFs are not correlated with estrogen levels. Estrogen has been shown to modulate the catecholamine levels within the hypothalamus, with the largest effect at the level of receptors (Etgen, Ansonoff, & Quesada, 2001). Specifically, estrogen induces down regulation of the presynaptic norepinephrine $\alpha_{2A/D}$ autoreceptor and upregulation of the α_{1B} postsynaptic receptor. Together, these alterations increase norepinephrine signaling by reducing negative feedback, and increasing postsynaptic availability of receptor biding sites (Karkanias, Ansonoff, & Etgen, 1996; Karkanias & Etgen, 1993). Further, catecholamines appear to be integral to the etiology of vasomotor symptoms. Before the onset of a HF, serum levels of epinephrine increase, while norepinephrine levels decreases (Cignarelli et al., 1989; Kronenberg et al., 1984; Kronenberg & Downey, 1987). Norepinephrine metabolites also become transiently elevated in plasma during HFs (Freedman, 1998).

Vasomotor symptoms are potentiated through activation of α2-adrenergic receptors and attenuated through blocking the same receptors. Administration of the α2-adrenergic antagonist, yohimbine, significantly increased the number of HFs recorded in symptomatic midlife compared to placebo administration (Freedman, Woodward, & Sabharwal, 1990). In addition,

administration of the α2-adrenergic agonist clonidine significantly increased the ambient temperature at which a HF would occur in symptomatic women (Freedman et al., 1990; Laufer, Erlik, Meldrum, & Judd, 1982; Tulandi, Lal, & Kinch, 1983). Notably, HFs were not induced in a group of midlife asymptomatic women with the administration or either yohimbine or clonidine (Freedman, 1998). The data suggest an underlying difference in α2-adrenergic signaling in women who experience vasomotor symptoms. There is additional evidence that the sympathetic nervous system is involved in HF etiology as indirect manipulation of the sympathetic nervous system through unilateral stellate ganglion block has been shown to reduce vasomotor frequency (Haest et al., 2012; Lipov & Kelzenberg, 2011; Lipov, Lipov, & Stark, 2005; Lipov et al., 2007; Pachman et al., 2011; Pachman, Jones, & Loprinzi, 2010).

iii. Menopause, HFs and Psychological Outcomes

HFs are associated with many negative health outcomes; however, women often present to their physician with complaints of vasomotor symptoms, sleep problems and memory dysfunction during menopause (Woods et al., 2000). Throughout the course of the menopausal transition, circulating levels of estrogens decrease dramatically, which has been implicated as a possible cause of changes in cognition, in particular memory (Maki & Hogervorst, 2003). Findings from the SWAN revealed a significant increase in complaints of "forgetfulness" when comparing women in the premenopausal stage (31%) to the perimenopausal (41%) and postmenopausal (44%) stages (Gold et al., 2000). In the Seattle Midlife Women's Health Study (SWMHS), 62% of women noted an undesirable change in memory functioning across the menopausal transition, and they most often attributed the decline in memory to stress, health issues, and aging (Woods et al., 2000). A follow-up study from this cohort revealed that forgetfulness related to difficulty concentrating, increases in follicle stimulating hormone (FSH),

and HFs (Woods et al., 2008). Rates of memory complaints in midlife women are high cross-culturally; in the Decisions at Menopause Study, over 45% of a sample of women living in Massachusetts, Madrid, and Beirut complained of memory loss, in addition to 34% of women living in Rabat, Morocco (Obermeyer & Sievert, 2007).

There is also evidence that subjective memory complaints relate to objective cognitive dysfunction in midlife women. A recent study in midlife women (n = 68) with frequent HFs found a link between current subjective memory complaints, measured by the Memory Functioning Questionnaire (MFQ)and memory performance on a list learning task (Drogos et al., 2013). Generally, mood and attention are related to memory complaints in midlife women (Drogos et al., 2013; Schaafsma, Homewood, & Taylor, 2009; Weber & Mapstone, 2009; Weber, Mapstone, Staskiewicz, & Maki, 2012); however there is conflicting evidence if objective memory performance is related to subjective complaints. Taken together, these data suggest that women can accurately predict changes in their memory abilities; however mood and menopausal symptoms may cause women to overestimate their cognitive dysfunction.

A relationship between cognitive performance and HFs was not demonstrated until objective measurement of HFs was incorporated into cognitive studies. Initial studies did not find any correlation between subjective HFs and cognitive performance (Ford et al., 2004; LeBlanc, Neiss, Carello, Samuels, & Janowsky, 2007; Polo-Kantola & Erkkola, 2004). A small observational study of 29 midlife women investigated the effects of objective and subjective HFs on cognition (Maki et al., 2008a). Objective HFs, but not subjective HFs, had a significant and negative relationship with verbal memory performance. Additionally, objective HFs during sleep were the strongest predictor of verbal memory performance when controlling for subjective sleep complaints. There was a significant and positive relationship between daytime objective

HFs and verbal fluency, and a trend for a positive relationship between measures of attention and objective HFs (Maki et al., 2008a).

Sleep complaints are common during the menopausal transition. Our previous research has suggested that physiological HFs during sleeping hours were the best predictor of memory dysfunction. Furthermore, subjective sleep quality did not change the relationship between nighttime HFs and memory. Sleep disruptions are commonly associated with decreases in memory performance (Backhaus et al., 2006), however our data suggests that objective HFs, not subjective sleep disturbance, was the cause of memory dysfunction in midlife women with moderate to severe vasomotor symptoms.

There is some evidence that self-reported HFs are associated with decreases in subjective sleep quality. Large epidemiological studies report that sleep disruption caused by HFs may be a contributing factor to sleep difficulties during the menopausal transition. Specifically, the frequency and severity of vasomotor symptoms were positively associated with subjective reports of the amount of time to fall asleep, ability to stay asleep, and the number of nighttime or early morning awakenings (Woods & Mitchell, 2010). In the SWAN, women with more frequent vasomotor symptoms were 4-5 times more likely to experience sleep problems compared with women without vasomotor symptoms (Kravitz et al., 2008). The largest sleep study in menopausal women to date, the Wisconsin Sleep Cohort Study (n = 589) found similar results. The presence of subjective HFs was associated with more dissatisfaction with sleep quality and daytime sleepiness; however, no differences were seen on PSG outcomes between the women who were and were not reporting HFs (Young, Rabago, Zgierska, Austin, & Laurel, 2003). Despite the lack of evidence linking objective sleep disruptions to vasomotor symptoms, there is a large body of evidence suggesting HFs may disrupt sleep structure. Disruptions in

sleep structure, or objective sleep quality may potentially be the mechanism of memory dysfunction in women experiencing frequent objective vasomotor symptoms.

Some of the earliest evidence that HFs were related to sleep came from a small observational study (n=19) which utilized polysomnography (PSG) and concurrently measured objective HFs. HFs during the night disrupted the normal progression of the sleep stages with each vasomotor event. This resulted in lower sleep efficiency and more time spent in slow wave sleep, but less time in REM sleep (Woodward & Freedman, 1994). Similarly, studies using PSG and objective HF monitoring found that awakenings are occurring before the onset of a HF (Freedman & Roehrs, 2004, 2006). The initial study (n= 19) investigated if HFs and awakenings were occurring simultaneously – or at different parts of the night. Most awakenings were occurring within the two 2 minutes before the onset of a HF and within lighter stages of non-REM sleep (Freedman & Roehrs, 2004). The follow-up to this study investigated if sleep disturbances of nighttime HFs were affected by ambient temperature and time of night (first versus second half of sleep). It was found that during the first half of the night, awakenings and changes in sleep stage occurred in the five minutes before a HF. During the second half of the night, when REM sleep is more prominent, awakenings were more frequently occurring within the five minutes after the HF. These data were taken as evidence that the suppression of thermoregulation during REM sleep may cause a reduction of HFs during the latter half of the night (Freedman & Roehrs, 2006). Furthermore, this was taken as evidence that the awakenings (and potentially the flashes themselves) were being caused though sympathetic arousal (Freedman, 1998; Freedman & Roehrs, 2006).

b. ANS

There is a scarcity of research focusing on the effect of menopause on the ANS; however, it has long been thought that the ANS is involved in vasomotor symptom physiology (Ginsburg et al., 1981). The transient increases in norepinephrine and epinephrine that occur after a HF may be a mechanism by which cognitive dysfunction or sleep disturbances are occurring in women who are experiencing vasomotor symptoms. In addition, the changes in ANS function associated with menopause and HFs can increase risk of cardiovascular disease. Recent research has shown a positive relationship between cardiovascular risk factors and cognitive impairment [Reviewed in: (Nash & Fillit, 2006)]. There is limited research directly investigating the effects of menopause and HFs on ANS function; however, there is a small body of research linking vasomotor symptoms to disrupted HRV (Hoikkala et al., 2010; Thurston et al., 2010, 2012). To our knowledge, there is no research investigating the effects of menopause or vasomotor symptoms on sAA activity. There is some evidence that crude, but clinically relevant, cardiovascular markers of sympathetic nervous system activity, like heart rate and blood pressure, show changes associated with menopause and vasomotor symptoms (Matsukawa et al., 1998; Owens, Stoney, & Matthews, 1993; Staessen, Bulpitt, Fagard, Lijnen, & Amery, 1989; Tuomikoski, Haapalahti, Sarna, et al., 2010; Tuomikoski, Haapalahti, Ylikorkala, et al., 2010; Zanchetti et al., 2005).

i. Effects of Menopause on the ANS: Focus on the Cardiovascular System

The ANS controls cardiovascular function through a balance between the excitatory sympathetic nervous system and the calming parasympathetic nervous system. Often, cardiovascular measurements like blood pressure, heart rate and HRV are used as a proxy measurement for sympathetic and parasympathetic activity. HRV, specifically high frequency

HRV, is a measure of parasympathetic input to the heart. Decreases in HRV parameters have been associated with increased risk of mortality associated with cardiovascular disease, confirming the validity of the measure (Bigger et al., 1992; Hillebrand et al., 2013; La Rovere et al., 1998; Wolf et al., 1978). A shift in the balance between sympathetic and parasympathetic drive may be contributing to an increased risk for cardiovascular disease and may also be the link between vasomotor symptoms and cognitive dysfunction.

The high frequency component of HRV represents the parasympathetic input into the heart. One component, respiratory sinus arrhythmia (RSA) specifically measures cardiac vagal tone. This projection originates from the nucleus ambiguous, and projects to the ventricles. Generally, there is strong evidence that RSA and high frequency HRV represent parasympathetic input to the heart. Specifically, vagal denervation in dogs results in a complete elimination of RSA, suggesting a primary role of vagal input (Chiou & Zipes, 1998). Blockade of the parasympathetic input to the heart using glycopyrrolate, a muscarinic agonist, resulted in elimination of the high frequency spectrum of HRV (Akselrod et al., 1985). Injection of both glycopyrrolate and propranolol, a β-adrenergic agonist, resulted in a flattening of all HRV spectrums. Individuals who undergo heart transplant surgery often maintain their atrial sinus node, while the ventricles of the donor heart are denervated. This results in a high resting heart rate, as there is no parasympathetic input to the donor heart. Moreover, there is a flattening of the HRV spectrum with an dramatic attenuation of high frequency HRV (Sands et al., 1989). Overall, data from drug trials and heart transplant data strongly suggest that high frequency HRV represents parasympathetic input to the heart.

Changes in sympathetic nervous system activity after menopause, are mediated though α-adrenergic peripheral vasoconstriction (Freedman, Sabharwal, & Desai, 1987; Mercuro et al.,

2000; Vongpatanasin, 2009; Weitz, Elam, Born, Fehm, & Dodt, 2001), and are associated with increased blood pressure (Vongpatanasin, 2009). There is evidence that estrogen levels may affect sympathetic activity in women. After menopause women are at higher risk for increased blood pressure irrespective of age or body mass index (BMI) (Owens et al., 1993; Staessen et al., 1989; Zanchetti et al., 2005). In addition, women with premature ovarian failure due to Turner syndrome, a chromosomal abnormality in which all or part of one of the sex chromosomes is absent, have increased blood pressure, when compared to age matched premenopausal controls (Gravholt et al., 1998). Sympathetic nervous system activity increases with age; however, the rate of change is much faster in women compared to men (Matsukawa et al., 1998; Narkiewicz et al., 2005). This is thought to be due to the gradual loss of circulating estrogens across the menopausal transition. There is some evidence that menopausal status has an effect on the increased rate of change in sympathetic activity in women as they transition through menopause (Matsukawa et al., 1998). Measures of sympathetic nerve activity (i.e. blood pressure and norepinephrine spillover) were decreased in perimenopausal women (n = 12) after estrogen therapy (oral estradiol valerate, 2 mg daily) but not after receiving placebo treatment (Komesaroff, Esler, & Sudhir, 1999).

Changes in ANS activity associated with menopause may be mediated through changes in estrogen signaling in the brain. The ANS controls heart rate and blood pressure through afferent and efferent input to the nucleus of the solitary tract through the vagus nerve. During menopause, decreased signaling from the vagus nerve to the nucleus of the solitary tract may result in decreased control of vascular dilation (Simonian et al., 1998). There is a high density of estrogen receptors in the nucleus of the solitary tract (Simonian et al., 1998) and these receptors affect adrenergic activity (Wang et al., 2006). Systemic administration of estrogen to

ovariectomized rodents can decrease blood pressure and heart rate (He, Wang, Crofton, & Share, 1998; Pamidimukkala, Taylor, Welshons, Lubahn, & Hay, 2003; Saleh & Connell, 2000).

Orthostatic stress is the stress associated with changes to the upright or standing body position. Upon standing there is an initial drop in blood pressure caused by gravity pooling blood into the lower limbs and this change in blood pressure is detected by to baroreceptors in large arteries such as the aortic arch, resulting in increased heart rate. The adrenergic system controls the initial increase in heart rate associated with an orthostatic challenge, such as moving from a seated to standing position. In addition, increases in sympathetic output cause vasoconstriction in skeletal muscle (Wallin & Sundlof, 1982). Increased sympathetic control of the heart can be achieved by either an increase in sympathetic tone, or through a decrease in parasympathetic tone. Decreased parasympathetic drive (the calming input to the heart) has been cited as one mechanism for increased cardiovascular risk after menopause.

It is important to note that increasing age has been associated with decreases in HRV parameters (O'Brien, O'Hare, & Corrall, 1986). Beyond studies of aging, there are few studies investigating the effects of menopause or hormone therapy on HRV in women; however, these few studies suggest that HRV decreases with menopause regardless of age. In addition, there is evidence that estrogen therapy can ameliorate those effects. In a small study (n = 20) of sedentary pre- and postmenopausal women in Brazil, postmenopausal women had significantly lower HRV parameters compared to premenopausal women during a supine and sitting orthostatic challenge (Ribeiro et al., 2001). These data suggest that being in the postmenopausal stage is associated with decreased parasympathetic input to the heart; however conclusions from these data are limited as age was confounded with menopausal status within this design.

The first study to investigate the effects of estrogen therapy on HRV, was a non-randomized trial of estrogen therapy (Epiestrol 50 mg patches; n = 18) versus a control group (n = 12) (Rosano et al., 1997). All subjects underwent a 24-hour heart rate monitoring session at baseline, one month after treatment, and four months after treatment. There were no significant differences in HRV at baseline, however at both one and four months after treatment all parameters of HRV showed a significant improvement (increase) in women on estrogen therapy (Rosano et al., 1997). Women in the control group maintained consistently lower h HRV (high and low frequency) from baseline though 4 months of treatment. This HRV profile is suggestive of increased sympathetic tone. These results also support the idea that decreasing estrogen levels are associated with an increase in sympathetic, but not parasympathetic input to the heart; however, interpretation of these data is limited as a non-randomized design can be confounded by self-selection bias.

The effects of estrogen withdrawal and replacement on HRV were thoroughly addressed in a cohort of 28 women undergoing either hysterectomy or hysterectomy with oophorectomy (Mercuro et al., 2000). Ambulatory 24-hour HRV was collected before surgery, four to five weeks after surgery, and after 3 months of estrogen therapy (in a subset of 10 women). After surgery, high and low frequency HRV were decreased only in the oophorectomy group, suggesting negative alterations in sympathetic and parasympathetic input to the heart associated with loss of circulating estrogen. Furthermore, three months of estrogen treatment ameliorated the dysfunction that was seen post-surgery (Mercuro et al., 2000). These results highlight the involvement of estrogen on the balance of the sympathetic and parasympathetic nervous system.

ii. Vasomotor Symptoms and ANS Function: HFs and Heart Rate Variability

Dysfunction in autonomic balance during the menopausal transition may be exacerbated in women who experience vasomotor symptoms. There is evidence that HFs may cause disturbances in autonomic balance beyond those associated with menopause alone. Specifically, HF may further shift the balance of the ANS toward more sympathetic activation. Higher diastolic blood pressure is associated with increased sympathetic activation (Louis, Doyle, & Anavekar, 1973; Louis, Doyle, Anavekar, & Chua, 1973; Philipp, Distler, & Cordes, 1978). Midlife women who report HFs have higher systolic and diastolic blood pressure compared to women who report never experiencing HFs (Gallicchio et al., 2010). In addition, a positive relationship was seen between HFs and blood pressure in a small observational study investigating 24-hour ambulatory blood pressure and heart rate. Specifically, severe nighttime HFs were associated with a transient increase in blood pressure and heart rate (Tuomikoski, Haapalahti, Ylikorkala, et al., 2010).

Previous research has shown interactions between cardiovascular health, estrogen therapy, blood pressure, and HFs (Hautamaki et al., 2012; Hautamaki et al., 2011; Hoikkala et al., 2010; Thurston et al., 2010, 2012). Loss of estrogen due to surgical menopause is associated with deregulation of the sympathetic-parasympathetic balance. In addition, estrogen therapy has been associated with a rebound of autonomic control of the heart and blood pressure (Mercuro et al., 2000). There is also evidence to suggest that hormone therapy may have differential effects on cardiovascular function depending on the presence or absence of vasomotor symptoms (Tuomikoski et al., 2009a, 2009b; Tuomikoski, Haapalahti, Sarna, et al., 2010; Tuomikoski, Haapalahti, Ylikorkala, et al., 2010). This supports the idea that women with HFs may have additional imbalance between the two branches of the ANS.

Most, but not all, previous research suggests that HFs are associated with a shift in the autonomic balance towards increased sympathetic drive to the heart. During HFs, transient decreases in high frequency HRV have been observed, suggestive of a withdrawal of parasympathetic activity rather than an increase in sympathetic activity. The incongruent findings with HRV and HFs may be partially explained by the difference in methods using subjective HFs compared to physiologically detected HFs. Previous studies have seen differing results when looking at the effects of subjective versus objective HFs (Maki et al., 2008a). All studies utilizing objective reported HFs saw a negative relationship between HRV and vasomotor symptoms, (Freedman et al., 2011; Thurston et al., 2010, 2012) while no relationship was seen in studies utilizing subjective HFs (Hautamaki et al., 2012; Hautamaki et al., 2011; Hoikkala et al., 2010; Lantto et al., 2012).

The first study to investigate the association between HRV parameters and vasomotor symptoms was an observational study of 150 postmenopausal women with mild, moderate, or severe self-reported vasomotor symptoms (Hoikkala et al., 2010). Overall, no relationship was seen between HRV outcomes and vasomotor symptom severity. During a HF, there was a significant decreased variability in normalized very low frequency, low frequency and, high frequency HRV when compared to control periods of time. The transient decreases in HRV parameters were replicated during laboratory stressors (Thurston et al., 2010) and during 24-hour ambulatory conditions (Thurston et al., 2012). These data suggest that during a HF there is a disruption of normal autonomic control of the heart. Specifically, decreased high frequency HRV is indicative of vagal withdrawal, or a decrease in parasympathetic input to the heart (Porges, 1991). Low frequency (Baselli et al., 1986) and very low frequency (K'Itney R, 1974; Taylor,

Carr, Myers, & Eckberg, 1998) are less understood but have been associated with both sympathetic and parasympathetic responses.

Vasomotor symptoms during the nighttime are associated with decreased memory functioning more than daytime HFs (Maki et al., 2008a). Changes in autonomic tone during nighttime HFs can be used to predict which HFs will result in awakening from sleep. A small observational study of 16 postmenopausal women investigated HRV during sleep, and utilized objective measures of both sleep and HFs. They observed that HFs that did not result in nighttime awakening caused decreased low frequency HRV or increased sympathetic tone. HFs that resulted in nighttime awakening had a slightly different HRV profile, with an initial decrease in low frequency HRV before the HF, but no change in low frequency HRV during or after the HF (Freedman et al., 2011). These data suggest that changes in sympathetic tone may simultaneously cause arousal from sleep and result in vasomotor symptoms.

Not all studies suggest that vasomotor symptoms have a negative effect on autonomic balance. A recent observational study enrolled 150 postmenopausal women with and without HFs to complete a cardiovascular and ANS assessment. Subjects completed a controlled and deep breathing task, active orthostatic test, Valsalva maneuver, and the handgrip test (Hautamaki et al., 2011). Women were grouped according to the severity of their subjective vasomotor symptoms: none, mild, moderate, and severe. No differences on heart rate, HRV, or blood pressure were observed; however, there was a significant decrease in the tachycardia ratio in women with vasomotor symptoms compared to those without (Hautamaki et al., 2011).

A randomized clinical trial investigated the effects of oral and transdermal hormone replacement therapy on 24-hour ambulatory HRV at baseline and after 6 month of treatment. A total of 150 midlife women participated (72 symptomatic; 78 asymptomatic). Women were

between six months and three years from their last menstrual period, and were randomized to receive one of the following treatments: transdermal estradiol, unopposed oral estradiol or oral estradiol combined with medroxyprogesterone acetate (MPA). At baseline, there were no differences in cardiovascular health between symptomatic or asymptomatic women. Compared to baseline, symptomatic women taking unopposed oral estradiol had decreased HRV during sleep. Asymptomatic women taking estrogen and MPA had decreased standardized intervals and time domain HRV. In addition, symptomatic women taking estradiol and MPA had more disruptions in their cardiac rhythm than women taking unopposed estrogen (Lantto et al., 2012). Overall, these data suggest that both symptomatic and asymptomatic women who were on hormone therapy with progesterone had a worse HRV profile after treatment.

c. sAA

sAA is an indirect biomarker of sympathetic nervous system activity (Nater & Rohleder, 2009). The sympathetic nervous system controls the body's ability to ready itself for "fight or flight" response. Among other things, activation of the sympathetic nervous system increases heart hate, contracts the pupils and blood vessels and increases sweating. Furthermore, activation of the sympathetic nervous system causes the release of sAA mainly from the parotid salivary gland (Rohleder & Nater, 2009).

It has long been thought that sympathetic activity is involved in the etiology of vasomotor symptom physiology (Ginsburg et al., 1981). Women with vasomotor symptoms show evidence of imbalance between the sympathetic and parasympathetic nervous system activity. Specifically, symptomatic midlife women have elevated basal blood pressure, suggestive of increased sympathetic drive (Gallicchio et al., 2010; Tuomikoski, Haapalahti, Sarna, et al., 2010; Tuomikoski, Haapalahti, Ylikorkala, et al., 2010), yet each HF is associated with a transient

decrease in heart rate and blood pressure (Germaine & Freedman, 1984). Two of the main neurotransmitters of the sympathetic nervous system, epinephrine and norepinephrine are altered following a HF (Cignarelli et al., 1989; Kronenberg, 1990; Swartzman et al., 1990). Measures of HRV also suggest an imbalance between the sympathetic and parasympathetic nervous system during a HF (Ford et al., 2004; Freedman et al., 2011; Thurston et al., 2010, 2012). High frequency HRV is a measure of respiratory sinus arrhythmia (RSA) or parasympathetic input to the heart (Akselrod et al., 1981; Katona & Jih, 1975; Porges, 2007). Low frequency HRV is associated with sympathetic nervous system activity (Pagani et al., 1986; Pomeranz et al., 1985); however components of parasympathetic activity may also represented in this measurement (Saul, Rea, Eckberg, Berger, & Cohen, 1990). The ambiguity regarding the interpretation of low frequency HRV requires additional markers of sympathetic activity.

The main neurotransmitters utilized by the sympathetic nervous system are acetylcholine and norepinephrine. Acetylcholine is released by the central nervous system to act upon sympathetic ganglion; in addition it activates sweat glands. Norepinephrine is released by postganglionic neurons to act on target tissues via adrenergic receptors. Circulating levels of norepinephrine show a strong positive relationship with sAA (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Thoma, Kirschbaum, Wolf, & Rohleder, 2012). In addition manipulation of adrenergic receptors results in changes in sAA levels. Activation of beta-adrenergic receptors (Chatterton et al., 1996) and alpha-2 adrenergic receptors cause increases in sAA (Ehlert, Erni, Hebisch, & Nater, 2006); likewise, blockade of beta-adrenergic receptors decrease levels of sAA (Nederfors & Dahlof, 1992; Speirs, Herring, Cooper, Hardy, & Hind, 1974; van Stegeren, Rohleder, Everaerd, & Wolf, 2006).

The sympathetic nervous system activity, specifically at the α-adrenergic receptors has been linked to the etiology of HFs. Early studies of non-hormonal treatments for HFs used clonidine, a centrally acting α-adrenergic antagonist. In a double-blind crossover study of 86 midlife women receiving 25-75 μg of clonidine, participants experienced an approximate 15% decrease in subjective HFs from baseline or placebo (Clayden, Bell, & Pollard, 1974). A follow-up dose-response study of clonidine (0.1-0.4 mg) on objective HF frequency reported up to a 46% reduction in objective HFs, as measured by skin resistance and finger temperature, (Laufer et al., 1982). However, of the ten participants, four withdrew due to negative side effects of the medication. The clonidine trial, in conjunction with the HRV studies (Freedman et al., 2011; Thurston et al., 2010, 2012) suggest an important involvement of sympathetic nervous system activity in HFs.

Levels of sAA are sensitive to stressors associated with sympathetic nervous system activity. Psychosocial stressors (Ehlert et al., 2006; Nater et al., 2010; Nater et al., 2006; Nater et al., 2005; Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004b) and physical exercise have been shown to reliably elevate sAA (Chatterton et al., 1996). A recent study of ninety healthy (84 women) young adults has also linked sAA with cognitive performance. Participants were exposed to a cold pressor test or a blanket control at encoding, consolidation, or retrieval of a word list (Smeets, Otgaar, Candel, & Wolf, 2008). Researchers found that sAA was positively associated with recall for those who received the stressor during consolidation. Conversely, sAA was negatively correlated with memory for those who received the stressor during retrieval (Smeets et al., 2008). These data suggest that increased sympathetic activity can enhance consolidation of information, but interfere with recall. Specifically, stress during learning and

consolidation may be beneficial to memory performance, while stress during retrieval can be detrimental to memory performance.

Generally, previous research supports the theory that the balance between the two branches of the ANS is altered in postmenopausal women compared to premenopausal women. In addition, there is evidence to suggest that HFs exacerbate this imbalance. This investigation aimed to determine if ANS function, measured by sAA and high frequency HRV, differed between women with frequent and infrequent HFs. The guiding hypothesis was that the relationship between HFs and ANS dysfunction would be more readily apparent when HFs are measured objectively than when assessed by self-report. An exploratory analysis focused on whether differences in ANS function may explain differences in psychological outcomes between the two groups of midlife women.

III. STATEMENT OF AIM AND HYPOTHESIS

This project aims to evaluate ANS function in women with and without frequent HFs by measuring HRV during an orthostatic challenge and sAA as markers of ANS activity. Additional exploratory aims focused on whether HF-related alterations in ANS activity contribute to the impact of HFs on psychological outcomes, including memory, sleep and mood.

General Hypothesis: The relationship between alterations in ANS function and HFs will be more apparent when HF are measured objectively using ambulatory skin conductance monitors than when measured subjectively using self-report.

Aim 1. To compare parasympathetic nervous system activity between women with and without frequent objective and subjective HFs.

Aim 1a. To compare parasympathetic nervous system activity between women with and without frequent *subjective* HFs.

Hypothesis 1a. High frequency HRV, a measure of parasympathetic nervous system activity, will not differ between women with frequent *subjective* HFs (≥ 30 per week) and women with few or no *subjective* HFs (≤ 7 per week). There will be a main effect of body position (supine, sitting and standing) on high frequency HRV such that HRV will have a linear decrease as stress on the heart increases, with the standing position having the lowest HRV and the supine position having the highest. There will be no interaction between body position and subjective HF group.

Aim 1b. To compare parasympathetic nervous system activity between women with and without frequent *objective* HFs.

Hypothesis 1b. Parasympathetic nervous system activity as measured by high frequency HRV during orthostatic challenge will differ between women with frequent *objective* HFs (≥ 30 per week) and women with few or no *objective* HFs (≤ 7 per week). Specifically, women with frequent objective HFs will show lower high frequency HRV compared to women with few or no objective HFs. The relationship between body position (Supine, Sitting, and Standing) and HRV will differ by objective HF group (Low vs. High Objective HF). Specifically, the magnitude of the group difference in high frequency HRV will be larger during the standing body position and smallest during the supine body position; indicative of increased vagal withdrawal in the standing position in women with High Objective HFs.

Aim 2. To compare sympathetic nervous system activity between women with and without frequent objective and subjective HFs.

Aim 2a. To compare sympathetic nervous system activity between women with and without frequent *subjective* HFs.

Hypothesis 2a. Sympathetic nervous system activity, as measured by sAA across the day, will not differ between women with frequent subjective HFs (≥ 30 per week) and women with few or no *subjective* HFs (≤ 7 per week). There will be a significant effect of time, with sAA levels dropping shortly after awakening and increasing across the day. There will be no interaction between time and *subjective* HF group (High vs. Low).

Aim 2b. To compare sympathetic nervous system activity between women with and without frequent *objective* HFs.

Hypothesis 2b. Sympathetic nervous system activity, as measured by sAA across the day, will differ between women with frequent *objective* HFs (≥ 30 per week) and women with few or no *objective* HFs (≤ 7 per week). There will be a significant main effect of time with higher levels of sAA later in the day. Finally, there will be a significant interaction between objective HF groups (low vs. high) and time. Specifically, there will be a larger decrease in sAA after waking in women with frequent *objective* HFs compared to women with few or no *objective* HFs, with no differences seen later in the day.

Exploratory Aim. To examine whether differences in ANS function are related to alterations in psychological outcomes, including memory, sleep and mood between women with frequent and infrequent HF.

IV. METHODS

This was an ancillary study of a larger multidisciplinary project examining potential mechanisms that might explain the relationship between HFs and memory dysfunction in a study called Cognition and Menopausal Symptoms (CAMS; L. Rubin, PI). A primary candidate mechanism in CAMS was cortisol. This ancillary study focused on ANS function in women with and without HFs, by investigating daily time course of sAA and HRV during an orthostatic challenge. The primary aim was to investigate if the number HFs (both subjective and objective) experienced across the day were related to changes in sympathetic or parasympathetic nervous system activity. Our secondary aim was to determine if ANS dysfunction, as measured by HRV and sAA activity, were also accompanied by changes in memory abilities. Inclusion and exclusion criteria were the same for both the parent study and the sub-study.

a. Participants:

Participants were recruited from the community through the use of fliers, Craigslist ads, and internal UIC electronic bulletin boards. Inclusion criteria were: 1) Women aged 40-65; 2) Women classified as perimenopausal or early postmenopausal based upon changes in cycle flow, cycle length and skipped periods; 3) intact uterus and at least one ovary; 4) able to give informed consent; English as first and primary language; and 5) moderate to severe self-reported HFs (i.e. defined by a standard criterion of > 30 HF per week) –OR- no to few self-reported HFs (i.e. <7 per week). Exclusion criteria were: 1) use of hormone therapies, selective estrogen receptor modulators (SERMS), phytoestrogens, or other pharmacological/botanical menopausal therapies within 2 months; 2) current smoking; 3) removal of uterus and ovaries; 4) use of antidepressants or sleep aids; 5) major systemic illness; 6) Axis I psychiatric disorder; 7) medical condition

affecting cognition; 8) use of prescription or over-the-counter medications affecting cognition; 9) first language other than English (because verbal memory is a primary outcome); 10) disorders or clinical conditions affecting cortisol levels; 11) diagnosis of sleep disorder; 12) cardiovascular disease; 13) concurrent participation in other clinical research studies; and 14) Body Mass Index > 33.

A total of 412 individuals were screened, of whom 130 qualified by phone screen. Of the women who qualified by phone screen, 88 completed a HF diary. A total of 31 women who completed a 2-week screening diary did not qualify due to not meeting inclusion criterion. The study involved a total of fifty-seven perimenopausal or postmenopausal women aged 40-60 (M = 52.36, SD = 4.95). A total of seventeen women are excluded from the analyses for the following reasons; biological data on thirteen subjects was lost due to a freezer failure, one woman only provided 4 hours of monitoring data, one woman did not provide saliva in her sampling tubes, one woman did not meet exclusion criterion when seen in person, and one woman did not complete a second visit. The final sample of 40 participants included 20 women who had more than 30 subjective HFs per week, and the other 20 had seven or fewer HFs per week as reported on a two-week prospective daily HF diary. Groups are matched on age and menopausal status which was defined using the STRAW +10 criteria (Harlow et al., 2012).

Demographics for the 40 study participants are presented in Table I. as a function of subjective HF group (Low or High) and in Table II. as a function of objective HF group (None or Any). Groups did not differ on age, race or menopausal stage. There were no significant differences between subjective HF groups (low frequency, high frequency) on any hormone or lipid variable. When splitting the data by objective HFs (none, any), no significant differences were observed between the objective HF groups. Seventy-eight percent of participants were not

taking any prescribed medication. The most common prescription medications were hydrochlorothiazide (n = 5), statin medication (n = 2), and omeprazole (n = 2). None of the participants were taking a beta-blocker medication.

Table I. Demographics for participants as a function of subjective hot flash group (Low Subjective Hot Flash and High Subjective Hot Flash).

	Hot Flash Diary Group			
Variables	Low Flash (n = 20)		High Flash (n=20)	
	M	*SD*	*M*	*SD*
Age	51.79	4.86	52.60	5.28
Education	16.63	1.83	16.35	2.35
Months since LMP	13.70	24.21	19.65	18.56
Cardiovascular Health Markers				
BMI	28.51	4.83	27.00	4.34
Systolic Blood Pressure†	129.10	16.76	119.55	16.29
Diastolic Blood Pressure	82.45	10.78	77.40	11.40
Triglycerides (mg/dL)	81.06	43.00	77.89	32.10
Total Cholesterol	189.67	33.66	205.58	38.43
LDL (mg/dL)	105.33	29.95	113.05	34.27
HDL (mg/dL)	69.28	19.75	77.42	23.83
KPAS Total	3.78	.69	3.75	.95
Hormone Values				
Estradiol	65.84	111.34	45.26	102.92
Follicular Stimulating Hormone	52.06	40.60	62.15	42.34
Race (%)				
Caucasian	31.6		35.0	
African-American	52.6		55.0	
Hispanic	10.5		5.0	
Other	5.3		0.0	
Menopause Stage (%)				
Peri-	63.2		40.0	
Post-	36.8		60.0	

Note: ** p< .001, *p<.01, † p <.10

Table II. Demographics for participants as a function of objective hot flash group (No Objective Hot Flash and Any Objective Hot Flash).

	Hot Flash Monitor Group			
Variables	No Flash (n = 13)		Any Flash (n=27)	
	M	*SD*	*M*	*SD*
Age	50.46	4.03	52.93	5.27
Education	16.08	2.14	16.63	2.06
Months since LMP	12.08	17.21	18.89	23.27
Cardiovascular Health Markers				
BMI	28.45	5.45	27.52	4.15
Systolic Blood Pressure	124.77	16.53	124.11	17.55
Diastolic Blood Pressure	80.38	12.82	79.70	10.66
Triglycerides (mg/dL)	80.31	41.81	78.24	34.98
Total Cholesterol	189.62	33.91	202.80	37.19
LDL (mg/dL)	108.54	34.72	109.52	30.17
HDL (mg/dL) †	65.08	13.29	77.56	24.20
KPAS Total	4.04	0.81	3.63	0.81
Hormone Values				
Estradiol	73.71	132.49	49.86	42.11
Follicular Stimulating Hormone	45.11	41.13	60.66	91.68
Race (%)				
Caucasian	30.8		33.3	
African-American	53.8		55.6	
Hispanic	15.4		3.7	
Other	0		7.4	
Menopause Stage (%)				
Peri-	61.5		48.1	
Post-	38.5		51.9	

Note: ** p< .001, *p<.01, † p <.10

b. General Procedures

Participants completed two visits which were conducted at the University of Illinois at Chicago Women's Mental Health Research Program. The first visit lasted about 2.5 hours and included a standardized neuropsychological test battery, a blood draw for estradiol and FSH levels, fitting and instruction with the Actiwatch and Biolog HF monitor (both devices detailed below) and instructions for saliva sample collection. Women were instructed to wear the HF monitor and Actiwatch for 72 hours and collect saliva samples at specified times over that same period. Approximately three days after the first visit, participants returned to lab for a second visit lasting about 1 hour. Participants completed a series of questionnaires to assess mood, anxiety and menopausal symptoms (see below) and completed an orthostatic challenge (see below). Participants were compensated a total of $125 for participating in both visits: $50 after the first visit and $75 after the second visit.

c. **Primary Psychophysiological Outcomes**

Objective HFs: For 72 hours, participants wore a validated and noninvasive ambulatory skin conductance monitor, the Biolog monitor (UFI, Model 3991x: Morro Bay, CA)(Carpenter et al., 1999). The monitor samples 12-bit skin conductance data at 1 Hz (once/second) from electrodes connected to the monitor by a 0.5 constant voltage circuit. Adhesive electrodes were applied using 0.05 M potassium chloride Unibase/glycol paste to record skin conductance. Participants were instructed to depress two buttons simultaneously on the monitor whenever they experienced a HF. This event was time-stamped and recorded with the continuous skin conductance on a memory card and was downloaded for analysis.

Hormone Assays: A venous blood sample was drawn at the beginning of the first visit by trained staff at the Center for Clinical and Translational Science Clinical Research Center (CCTS) at the

University of Illinois at Chicago. Blood was drawn into a serum separator tube, was immediately spun, and sent to Alverno Laboratories in Hammond, Indiana. Blood serum enzyme linked immunoassays of FSH and estradiol were run on all but one subject, due to laboratory error. The lower detection level for estradiol is 5 pg/mL and for FSH is 0.100 mIU/mL. Intra- and inter-assay coefficients of variance were less than 5% on both FSH and estradiol. These hormone levels were used to help validate menopausal stage.

Salivary Alpha-amylase: Salivary measures of sAA were obtained using a passive drool method. During a 24-hour period participants provided eight samples across the day into sterile Nalgene containers (Waking, +30 min, +3 hours, +6 hours & +12 hours after waking). We analyzed the SAA content in five of the eight samples (Waking, +30 min, +3 hours, +6 hours & +12 hours after waking). sAA was quantified at Salimetrics using a kinetic reaction assay (State College, PA). Area under the curve (AUC) with respect to ground (i.e. zero) was computed for each participant. We did not use the AUC with respect to baseline, as we were expecting a drop in sAA compared to the initial sAA. Awakening response of sAA was calculated with the following formula [(sAA +30 mins) – (sAA Wake)].

Heart Rate Variability: HRV was measured using a 3991x Biolog monitor (UFI, Morro Bay, CA) that was configured specifically to record millisecond inter-beat interval (IBI) from an ECG signal. Three ECG electrodes were placed on the participant's torso and connected to the Biolog through a Fetrode input assembly. Each participant wore the monitor for the duration of the orthostatic challenge. The data were downloaded via 3991 DPS software (UFI) to a computer for quantification of vagal regulation of the heart (i.e., the amplitude of RSA) and heart period. Data were segmented into separate conditions using data markers placed at the beginning and end of each condition. Heart period data for each condition were visually examined and outliers

were edited with CardioEdit software (Brain-Body Center, UIC). Heart rate, high frequency and low frequency HRV were calculated for each condition with CardioBatch software (Brain-Body Center, UIC) in accordance with standard procedures (Porges, 1985).

High Frequency HRV – Respiratory sinus arrhythmia (HF, 0.12-0.4 Hz) represents the parasympathetic input to the heart by the vagus nerve (Akselrod et al., 1981; Katona & Jih, 1975; Pomeranz et al., 1985; Porges, 2007), which is responsible for slowing the heart.

Low Frequency HRV– (LF, 0.06-0.10 Hz) The exact role of low frequency is unclear; however, previous research suggests it is related to sympathetic activity (Pagani et al., 1986; Pomeranz et al., 1985), baroreceptor activity (Akselrod et al., 1981; Sleight et al., 1995), and thermoregulation (Fleisher et al., 1996; K'Itney R, 1974). Low frequency HRV may also contain some information about parasympathetic nervous system activity (Saul et al., 1990).

Orthostatic Challenge Protocol: Participants were asked to stay in three different body positions for 5 minutes each during the second visit. The first position was a supine posture, with ankles uncrossed. Next participants were instructed to slowly move into a comfortable, but upright, seated position, with feet flat on the floor. Finally, participants were asked to remain standing. At the end of each position, blood pressure was taken. HRV outcomes were collected continuously throughout the challenge. The beginning and end of each body position data were demarcated within the data stream for editing as HRV data from each body position was edited and analyzed separately.

Cardiovascular Health Markers: Various predictors of cardiovascular disease risk were collected at the beginning of the first visit. Waist, hip and neck girth were measured using a

standard pliable tape measure. These three measurements in inches were used to estimate body fat percentage using a U.S. Navy algorithm [% body fat = 163.205 x log10(waist + hip - neck) - 97.684 x log10(height) - 78.387] (Defense, 2002). Height and weight were collected to compute the measure of Body Mass Index (BMI). Blood pressure was measured after participants had been seated in a relaxed position for 5 minutes.

d. **Primary Psychological Outcomes**

i. Neuropsychological Test Battery

The standardized neuropsychological battery took approximately one hour to complete. The primary outcome of paragraph recall was chosen based upon our previously published study which found a negative relationship between objective but not subjective HFs and memory performance. Moreover, tests of verbal fluency were selected as a positive trend between objective HFs and executive function was previously reported (Maki et al., 2008a). The Finding A's task was included because there was a trend toward a significant negative relationship between objective HFs and this measure in midlife women (unpublished data). Tests of visuospatial abilities were included as our previous research has shown a negatively relationship between HFs and visuospatial ability in men undergoing androgen deprivation therapy for prostate cancer (Jamadar et al., 2014). The Digit Span and the Brief Test of Attention were chosen, as they related to poorer sleep quality in our previous publication (Maki et al., 2008a) .

ii. Cognitive Outcomes

1. *Logical Memory Subtest of the Wechsler Memory Scale-Revised (WMS-R/LM-R) (Wechsler, 1981):* This is a test of both immediate and delayed recall of a short story. Participants are read a brief story which contains 25 discreet chunks of information. Participants are instructed that they would have to recall this story immediately, and

again at a later time (30-minute delay). Outcome measures included standardized scores of story recall accuracy both immediately after presentation and after a 30-minute delay, where the total scores range from 0 to 25.

2. *Modified Card Rotations Test* (Wilson et al., 1975): This is a timed test of visuospatial performance. Participants are given three minutes to view a series of twenty-eight line drawing geometric figures, followed by 8 alternatives which are either a 2- or 3-dimensional rotation of the target. Participants are instructed to choose and mark on the paper in the box beside and "S" for same which representations of the target are a rotation and mark on the paper in the box beside and "D" for different for those which are mirror-image representations of the target image. The outcome is the total number of correctly identified responses minus the number of incorrect responses across two series of 28 figures, with a maximum possible score of 160.
3. *Letter Fluency (Benton, 1968)* (Benton, 1968): This is a test of verbal fluency. Participants are given one minute to generate as many words as possible which begin with a particular letter. Participants are instructed to avoid saying proper nouns or to use the same word with multiple endings (e.g., dance, danced and dancing). The outcome is the total number of words produced across three trials.
4. *Digit Span Forward and Backward* (Wechsler, 1981): This is a test of both attention and working memory. During the Digit Span Forward test, the examiner reads a series of number strings to the participant, who is instructed to repeat the series back to the examiner. In Digit Span Backwards, the participant is read a string of numbers, and is asked to say the string back in reverse order. The outcome measures are the number of trials correctly completed for the forward and backward trials, respectively.

5. *Finding A's Test* (Ekstrom, French, & Harman, 1976): This is a test of visuoperceptual speed and attention. Participants are given a set of four papers containing five columns of words on each page. Participants are instructed to cross out the five words in each column that contain the letter 'A' as quickly and accurately as possible within 2 minutes. The outcome is the total number of correct responses.
6. *Brief Test of Attention (Schretlen, Bobholz, & Brandt, 1996):* This is a test of auditory attention. Participants are presented with a series of twenty trials, where participants hear a series of letters and numbers (e.g. 5-H-T). For one block of 10 trials, participants are told to track and report the number of letters presented, and in the other block of trials they are told to track and report the number of numbers presented. Difficulty increases as the series of numbers and letters increases from 4 to 18 items across the 10 trials. The outcome is the total number of correct responses. Lower performance on this test was associated with poorer subjective sleep in our previous study (Maki et al., 2008b).

iii. <u>Other Psychological Outcomes</u>

1. *Greene Climacteric Scale (GCS)*(Greene, 1998): This is a self-report questionnaire that measures four categories of menopausal symptoms, including psychological symptoms, somatic symptoms, vasomotor symptoms and sexual dysfunction. The questionnaire contains 21 items that are scored on a 4-point Likert scale (0 = "not at all" to 3 = "extremely"). Scores for each sub-scale was computed by summing the responses for each category.
2. *Pittsburgh Sleep Quality Index (PSQI)* (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989)*:* This is a self-report questionnaire that measures sleep quality, including the latency to fall asleep, sleep duration and sleep disturbances. Higher numbers indicate

greater sleep disturbance. A total sleep score was calculated using a previously published scoring scheme, with higher scores indicating greater sleep disturbance (Buysse et al., 1989).

3. *Positive and Negative Affect Scale (PANAS)* (Watson, Clark, & Tellegen, 1988): This is a 20-item self-report questionnaire measuring positive and negative mood states. Participants rate each mood state using a 5-point Likert scale based on the amount they experienced each state during the previous 7 days. Ratings for each item range from 1 to 5, with 1 indicating "very slightly" and 5 indicating "extremely." The outcomes are the total score for positive and negative mood states which are calculated separately. There is a maximum score of 50 for each scale.
4. *Perceived Stress Scale (PSS)(Cohen, Kamarck, & Mermelstein, 1983):* The PSS is a 10-item questionnaire developed for measuring the degree to which situations in one's life are perceived as stressful. Items were chosen to determine how "unpredictable, uncontrollable, and overloading" respondents found their lives to be and are rated on a 0 to 7-point Likert scale ranging from "Never" to "Very Often." A total score is obtained by reversing the scoring on five items, and then summing all of the responses. The outcome from this scale was used as a potential covariate.
5. *Beck Anxiety Inventory (BAI):* This self-report measure consists of 21 items measuring common anxiety symptoms (i.e., nervous, shaky). The respondent is asked to rate how much she has been bothered by each symptom over the past week on a 4-point scale ranging from 0 to 3. The outcome is a total sum of all items.
6. *Center for Epidemiological Studies, Depression Scale* (CES-D)(Radloff, 1977): The CES-D is a self-administered 20-item questionnaire which asks respondents how often

they have experienced certain depressive symptoms (e.g., feeling sad, lonely) in the past seven days. The questionnaire is administered on a Likert-like scale with participants endorsing each symptom occurring "never or rarely" (score of 0) to "most or all of the time" (score of 3).

7. *Kaiser Physical Fitness Questionnaire: Sports and Exercise subscale (Ainsworth, Sternfeld, Richardson, & Jackson, 2000):* This questionnaire asks participants to answer 15 questions regarding how often they exercise, how much exertion they give and what types of activities they participate in. A total activity score was computed using previously published scoring parameters (Sternfeld, Ainsworth, & Quesenberry, 1999). The outcome from this scale was used as a potential covariate for HRV outcomes as it is known that exercise affects HRV outcomes (Gregoire, Tuck, Yamamoto, & Hughson, 1996; Jurca, Church, Morss, Jordan, & Earnest, 2004).

V. STATISTICAL ANALYSIS

Prior to analyses, we examined the distribution of each outcome to ensure normality and check for statistical outliers (i.e., values >3SD above or below the mean). Raw values of sAA had a non-normal distribution; therefore data was transformed using natural logarithm of each value. For statistical outliers, we substituted the outlier value with the next most extreme non-outlier value in the variable distribution (Tabachnick & Fidell, 2001). There were 10 statistical outliers within measures of sAA (5% of the data). After the substitution, all sAA variables were normally distributed. There were no statistical outliers within measures of HRV, however 8% of the data were missing and we used a mean substitution, by subjective HF group, for missing data.

To reduce the number of comparisons, two z-scores were created for memory and attention domains, as done in previous studies (Hampson, 1990; Maki, Rich, & Rosenbaum, 2002; Mordecai, Rubin, & Maki, 2008). For each domain score, z-scores were calculated for each individual test across groups, and then averaged.The domain score for attention included the total scores for the Brieft Test of Attention, Digit Span Forward and Finding A's. The memory domain score included Logical Memory Immediate Recall and Logical Memory Delay Recall scores. Area under the curve (AUC) for the natural logged values of sAA was calculated for each subject based on the trapezoidal method(Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). Sensitivity was calculated for the HF data collected during the 72-hour monitoring session, with the following formula [True Positive HF/ (True Positive HF + False Negative HF))*100]. Demographic characteristics were compared between groups using

chi-square analyses for categorical variables or t-tests. All statistical comparisons were run using SPSS version 20, unless otherwise noted (IBM, Chicago, IL).

The first aim of this investigation was to compare parasympathetic nervous system activity, as measured by high frequency HRV, between women with frequent and infrequent subjective and objective HFs. To examine group differences on HF frequency group on HRV measures, we conducted a series of mixed-effects regression (MRM) analyses. Independent predictors included in the analysis were HF group (Subjective: none-to-mild vs. moderate-to-severe; Objective: none vs. any) and dummy variables for Body Position (Supine, Sitting and Standing), as well as all two-way interactions (i.e. group differences at each body position). We focused on group differences at each time point. MRM analysis was chosen as it has a flexible covariance structure, allowing for differential correlation among the repeated measures. Separate analyses were run for objective monitor group and subjective diary group. BMI, self-reported physical activity and waist to hip ratio were considered as a potential covariate for HRV outcomes. Only BMI was significantly correlated with the high frequency HRV outcomes, however inclusion of BMI in the model did not alter the results, therefore it was excluded in the final model. Given the small sample sizes, Cohen's d effect sizes are also reported (small effect = 0.2; medium effect = 0.5; large effect = 0.8) (Cohen, 1992). SAS statistical software version 9.2 (SAS Institute Inc, Cary, NC) was used for MRM analyses. Significance was set at $p < 0.05$.

We predicted a main effect of body position, such that there would be a decrease in HRV as stress level on the heart increases, with the greatest difference between supine and standing. We did not predict a main effect of diary group but did predict a main effect of objective HF group, such that women with frequent objective HFs would show lower high frequency HRV compared to women with low objective HFs. This pattern would be indicative of more vagal

withdrawal in women with frequent objective HF. We also predicted a main effect of body position, such that there would be a linear decrease in HRV as the body position becomes more strenuous on the heart and baroreflex (supine, sitting and standing). Finally, we predicted an interaction between body position and group. Specifically, we predicted that the difference between groups would be most prominent for HRV during the standing condition of the orthostatic challenge, as it is the most stressful on the heart.

The second aim was to measure the impact of HFs on sympathetic nervous system activity by comparing sAA across the day in women with frequent and infrequent subjective and objective HFs. The statistical approach which was used in the first aim was also taken for the second aim. A series of MRM analyses was conducted to examine differences on the independent predictors including HF group (Subjective: none-to-mild vs. moderate-to-severe; Objective: none vs. any) and the dummy-coded variables for Time (wake, +30min, +180min, +360min, and +720min). Again, Cohen's d effect sizes are also reported (Cohen, 1992).

We predicted a main effect of time, such that the levels of sAA would be higher in the later samples compared to the samples at wake. We also predicted a main effect of time such that levels of SAA would be higher in the afternoon and evening samples compared to the morning samples. We did not predict a main effect of subjective HF group, but did predict a main effect of objective HF group such that women with objective HF would have higher sAA compared to women without objective HF. We did predict an interaction between time and group, such that women with objective HFs would have a larger drop in sAA levels after wake compared to women with few or no objective HFs.

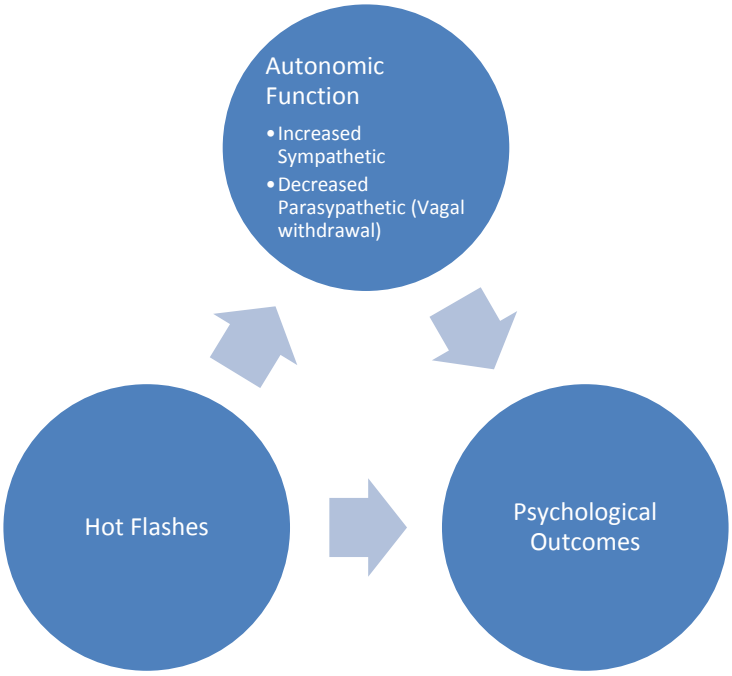
For the exploratory aim, correlations and multivariate regressions were used to determine the extent to which ANS outcomes were associated with objective and subjective HF, as well as

cognitive (i.e., attention and memory domain scores) and psychological outcomes. When warranted by correlational analysis ($p < .10$), autonomic variables (i.e. HRV outcomes, AUC or waking response of sAA) were used to predict primary psychological domains and HF frequency. Each outcome was analyzed in a separate stepwise regression equation. Further analysis to explore mediation models will be performed if there are significant relationships within the Pearson correlations ($p < .05$) between a series of all three outcome measures, HFs, ANS function and cognitive or psychological outcomes. For example, if we see significant relationships between objective HFs, awakening response of sAA, and anxiety as measured by the BAI we would complete a series of two forced entry linear regression models. The first linear regression would use objective HF frequency to predict awakening response of sAA. The second forced entry model would be use both objective HF frequency and awakening response of sAA to predict anxiety as measured by the BAI. Specifically, the beta-weights for each of these regression models will be used to determine if there is a mediational relationship, (as depicted in Figure 2.) using an effect decomposition model to calculate the direct and indirect effects of HF and ANS activity outcomes on psychological outcomes (Hafeman, 2009).

To determine if ANS activity was related to psychological outcomes, a series of Pearson's correlations and regressions were conducted between ANS outcomes (i.e. AUC or waking response of sAA) and memory and attention domain scores. Memory and attention domain scores were chosen due to previously reported relationships between these cognitive domains and sympathetic activity (Cahill & Alkire, 2003; Cahill & McGaugh, 1998; Eldar, Cohen, & Niv, 2013; Luu & Posner, 2003). PSS, BAI and CES-D outcomes were included in the correlation analyses because of previously reported relationships between chronic stress and mood and the diurnal rhythm of sAA (Nater et al., 2006). If warranted by the correlational analysis ($p > .10$), a

stepwise linear regression model will be run with the predictors (e.g. psychological or HF outcomes) which were related to AUC or waking response of sAA.

Figure 2. Conceptual model of the relationship between Autonomic function, Hot flashes and Psychological Outcomes.



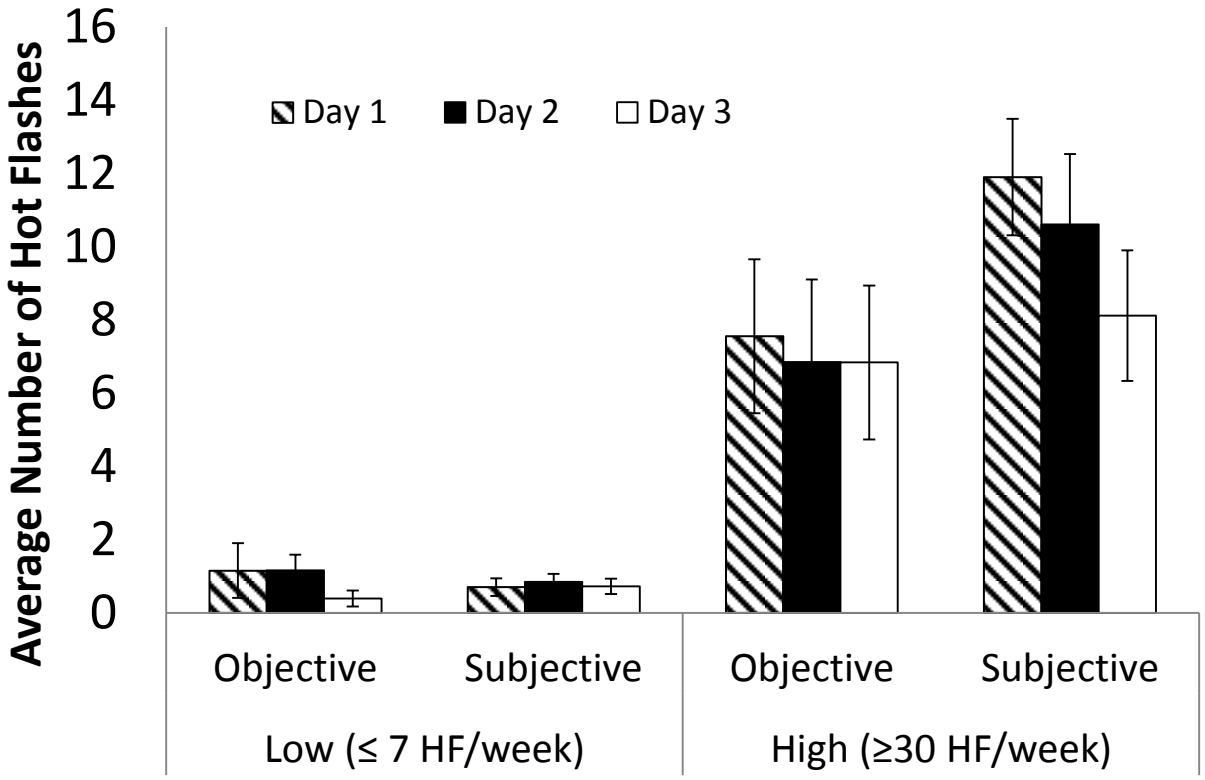
VI. RESULTS

a. Primary analyses

i. Subjective and Objective HFs

Figure 3. shows the frequency of subjective and objective HFs by subjective diary group (low versus high). As expected, women in the high frequency subjective HF group reported more HF (mean = 125.00) than women in the low frequency subjective HF group (mean = 4.35) in the two-week screening diary ($F(1, 39)= 86.57$, $p < .001$). Due to an unexpected high frequency of women with no or few (≤ 3) objective HFs across the 72-hour monitoring session (n = 9, 45%), the data were negatively skewed, and therefore the objective HF groups were women with no objective HFs (n=13) versus women with any objective HFs (n = 27; mean objective HF in 24 hours = 6.45). The 13 women with no objective HF included ten women in the low subjective HF group and three in the high subjective HF group. In addition, a total of three women classified in the high frequency subjective HF group did not have any objective HFs. The 27 women with objective HF included six women in the low subjective HF group and 11 in the high subjective HF group. Of the 20 women in the low subjective HF group, 10 had no objective HFs during the monitoring session and nine had any HFs. Of the 20 women in the high frequency subjective HF group three had no objective HFs, and 17 had at least one. Finally, nine women who were originally classified in the high frequency subjective HF group failed to meet the threshold of 4.3 HF within the 72-hour monitoring session.

Figure 3. Subjective and Objective Hot Flashes by Day as Function of Subjective Hot Flash Group (Low Subjective Hot Flash and High Subjective Hot Flash).



Four subjects did not have valid objective HF data for a complete 72 hours; three subjects had 48 valid hours of data and one subject had 24 valid hours. To maximize the sample size, all analyses were completed using the data from the first 24-hours of data collection. To ensure that the first day of data collection was representative of the whole 72-hour session we examined correlations between the frequency of objective HF on Days 1, 2, and 3 of the monitoring period, and also compared the mean frequencies and sensitivity (number of objective HFs subjectively detected) across the three days. There was a significant positive correlation between all days of data collection ($p < .001$); coefficients ranged from .71 to .92 and are shown in Table III. Average frequencies of subjective and objective HFs, by diary group, are depicted in Figure 3. Frequency of both objective and subjective HFs were significantly higher in the high frequency HF diary group compared to the low frequency HF diary group across all three days of monitoring (p's $< .02$). Sensitivity did not significantly differ between subjective HF groups across the three days of data collection ($p > .05$). Mean sensitivity data is presented in Figure 4.

Figure 4. Sensitivity by Day as Function of Subjective Hot Flash Group (Low Subjective Hot Flash and High Subjective Hot Flash).

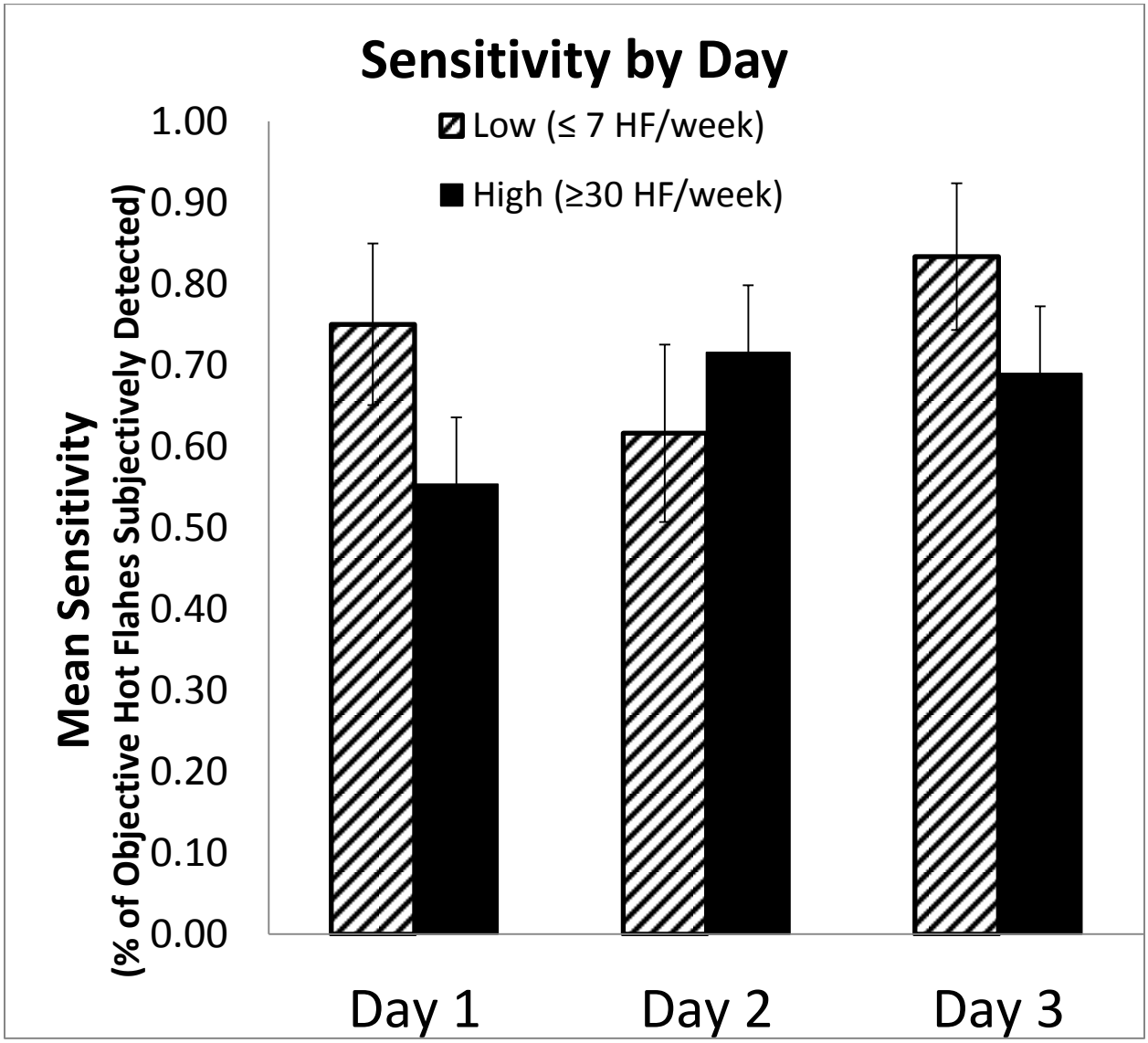


Table III. Correlation Coefficients for Subjective and Objective Hot Flash Data across the 72 Hours of Data Collection.

	Day 1	Day 2	Day 3
Objective Hot Flashes			
Day 2	.92**		
Day 3	.73**	.85**	
Subjective Hot Flashes			
Day 2	.83**		
Day 3	.71**	.88**	
Note: ** p< .001, *p<.05, † p <.10			

ii. Cognitive Outcomes

Table IV shows the cognitive outcomes for each subjective HF group. Cognitive performance did not vary as a function of subjective HF group (*p's* > .08). Table V shows the cognitive outcomes as a function of objective HF group. No significant differences in cognitive test performance were seen between women with or without objective HFs (*p's* > .16).

Table IV. Summary of Cognitive Outcomes as a Function of Subjective Hot Flash Group (Low Subjective Hot Flash and High Subjective Hot Flash).

Variables	Hot Flash Diary Group			
	Low Flash (n = 20)		High Flash (n=20)	
	M	*SD*	*M*	*SD*
Memory				
Logical Memory Immediate	10.37	2.22	10.40	3.28
Logical Memory Delayed	9.47	3.04	8.95	3.78
Digit Span Forward	8.58	1.77	8.30	2.23
Digit Span Backward	6.84	2.48	6.35	2.23
Brief Visual Retention Test	7.0	1.86	6.40	2.16
Visuospatial				
Card Rotations	65.89	41.11	57.80	33.44
Attention				
Finding A's	31.26	8.75	27.95	6.65
Brief Test of Attention	14.74	3.16	14.20	2.88
Fluency				
Verbal Fluency†	48.37	10.60	41.80	10.54
Semantic Fluency	48.32	11.15	47.30	9.10

Note: ** p< .001, *p<.05, † p <.10

Table V. Cognitive Outcomes for as a Function of Objective Hot Flash Group (No Objective Hot Flash and Any Objective Hot Flash).

Variables	Hot Flash Diary Group			
	No Flash (n = 13)		Any Flash (n=27)	
	M	SD	M	SD
Memory				
Logical Memory Immediate	9.54	1.81	10.63	3.18
Logical Memory Delayed	8.38	2.22	9.52	3.83
Digit Span Forward	8.31	1.80	8.52	2.08
Digit Span Backward	6.62	2.26	6.52	2.39
Brief Visual Retention Test	6.15	1.82	6.89	1.97
Visuospatial				
Card Rotations	66.31	40.69	57.81	36.16
Attention				
Finding A's	30.85	9.15	28.81	7.06
Brief Test of Attention	13.62	3.48	15.04	2.75
Fluency				
Verbal Fluency†	47.92	8.71	43.26	11.67
Semantic Fluency	48.00	7.96	47.70	10.84

Note: ** p< .001, *p<.05, † p <.10

iii. Psychological Outcomes

Table VI shows the psychological outcomes by subjective HF group. Compared to women in the low frequency subjective HF group, those in the high frequency subjective HF group had significantly greater levels of anxiety symptoms as measured by the BAI ($F(1, 39)= 5.94$, $p= .02$, $d = -0.78$) , reported more menopausal symptoms as reported by the Greene Climacteric Scale (GCS; $F(1, 39)= 10.05$, $p= .003$, $d= -1.02$), and reported significantly worse sleep quality, as measured by the PSQI ($F (1, 39)= 12.64$, $p= .001$, $d= -1.15$). Table VII shows the psychological outcomes by objective HF group. In contrast to the analyses of subjective HF group, analyses of psychological outcomes by objective HF frequency, there were no significant differences between women who experienced objective HFs and those who did not.

Table VI. Psychological Outcomes as a Function of Subjective Hot Flash Group (Low Subjective Hot Flash and High Subjective Hot Flash).

	Hot Flash Diary Group			
Variables	Low Flash (n = 20)		High Flash (n=20)	
	M	SD	M	SD
Quality of Life				
Greene Climacteric Scale*	8.06	6.88	16.85	10.30
Psychological*	4.97	3.95	9.0	5.86
Somatic†	2.20	2.78	4.20	3.76
Vasomotor**	0.90	1.25	3.65	1.81
Utian Quality of Life	82.60	13.10	88.45	8.34
Hot Flush Beliefs Scale	32.20	18.72	41.66	20.78
Mood				
Beck Anxiety Index*	7.95	6.49	14.20	9.45
Anxiety Sensitivity Scale	18.05	11.70	21.10	11.87
Perceived Stress Scale	15.30	5.92	14.30	8.06
CES-D†	11.80	7.49	16.45	9.83
Affect				
PANAS Positive Affect	35.85	8.58	36.30	9.35
PANAS Negative Affect†	16.15	5.58	19.90	7.28
Sleep				
Pittsburg Sleep Quality Index**	6.00	2.71	9.95	4.16

Note: ** p< .001, *p<.05, † p <.10

Table VII. Psychological outcomes as a Function of Objective Hot Flash Group (No Objective Hot Flash and Any Objective Hot Flash).

Variables	Hot Flash Diary Group			
	Low Flash (n = 13)		High Flash (n=27)	
	M	SD	M	SD
Quality of Life				
Greene Climacteric Scale	12.30	9.41	12.53	10.05
Psychological	7.45	5.27	6.76	5.46
Somatic	3.38	3.69	3.11	3.36
Vasomotor†	1.46	1.56	2.67	2.20
Utian Quality of Life†	80.69	11.60	87.85	10.49
Hot Flush Beliefs Scale	34.50	16.29	36.24	22.09
Mood				
Beck Anxiety Index	10.15	7.46	11.52	9.21
Anxiety Sensitivity Scale†	15.15	10.40	21.70	11.92
Perceived Stress Scale	15.54	6.41	14.44	7.36
CES-D†	13.08	8.884	14.63	9.09
Affect				
PANAS Positive Affect	36.08	8.76	36.07	9.08
PANAS Negative Affect	17.46	6.36	18.30	6.92
Sleep				
Pittsburg Sleep Quality Index†	6.31	3.12	8.78	4.18

Note: ** p< .001, *p<.05, † p <.10

iv. HRV

Consistent with our predictions, when examining the effect of subjective HF and body position on HRV, there was a significant main effect of body position on high frequency HRV. High frequency HRV was significantly lower in the standing body position when compared to both supine (β= 0.38, *SE*= 0.14, *p*= .007, *d*= 1.20) and sitting (β= 0.59, *SE*= 0.14, *p*< .0001, *d*= 1.86.) positions. This overall effect of body position confirms that our orthostatic challenge manipulation was successful. As predicted, there was no overall difference in high frequency HRV by subjective HF group (β= 0.23, *SE*= 0.27, *p*= .41, *d*= -0.41). Additionally, there were no significant differences in HRV between subjective HF groups at any of the three individual body positions (*p's*> .36) (see Figure 5).

When the data were analyzed by objective HF group, a significant effect of body position was again observed. , Specifically, high frequency HRV was significantly lower in the standing compared to supine (β= 0.43, *SE*= 0.14, *p*= .004, *d*= 0.58) and sitting body positions (β= 0.57, *SE*= 0.14, *p*< .001, *d*= 0.77). Contrary to our hypothesis, we did not observe a main effect of objective HF group on high frequency HRV, or any significant differences in HRV between objective HF group at any of the three individual body positions (*p's*> .22) (see Figure 6).

Figure 5. Average High Frequency HRV across Orthostatic Challenge Body Positions by Subjective Hot Flash Group (Low Frequency vs. High Frequency).

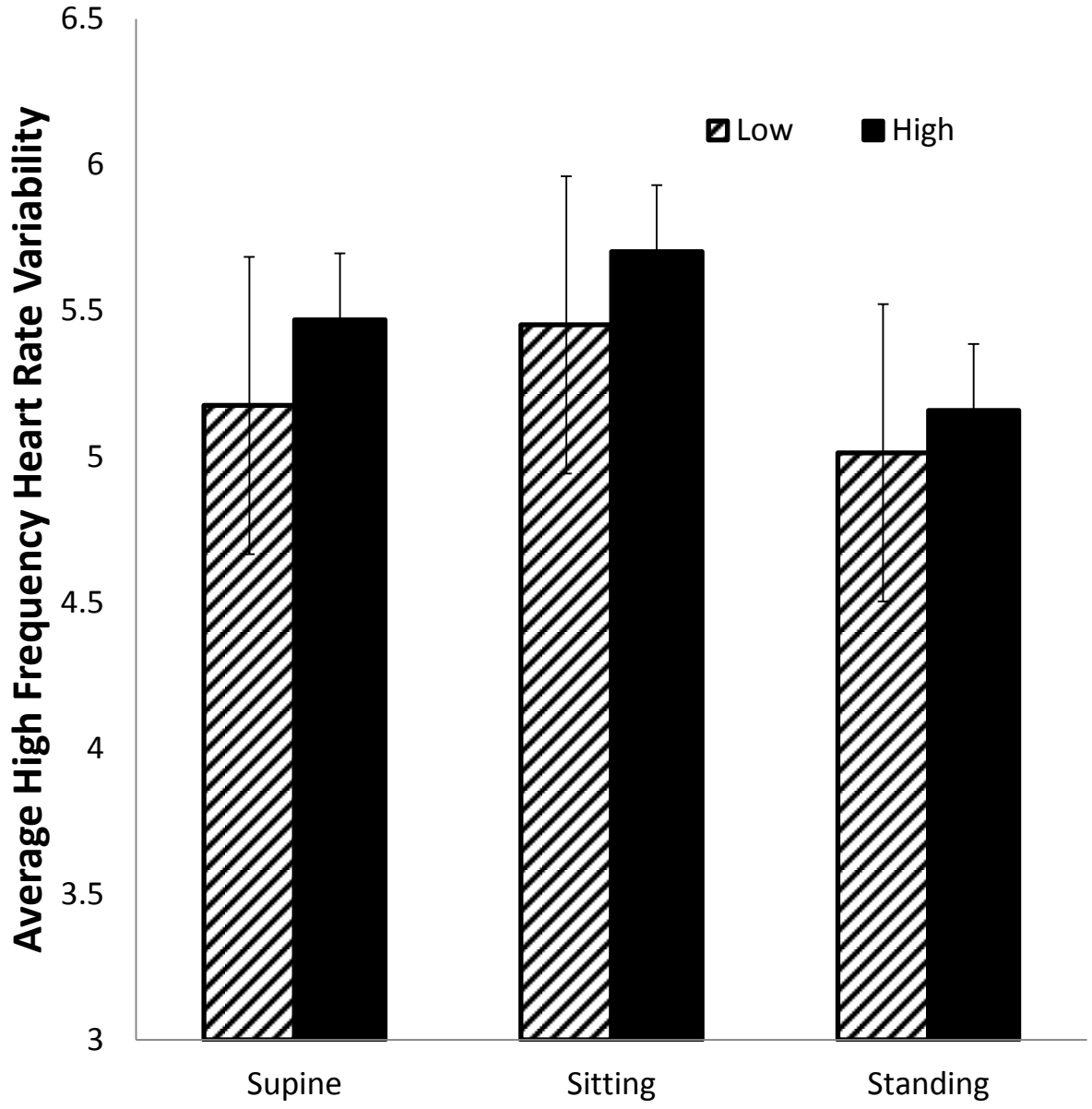
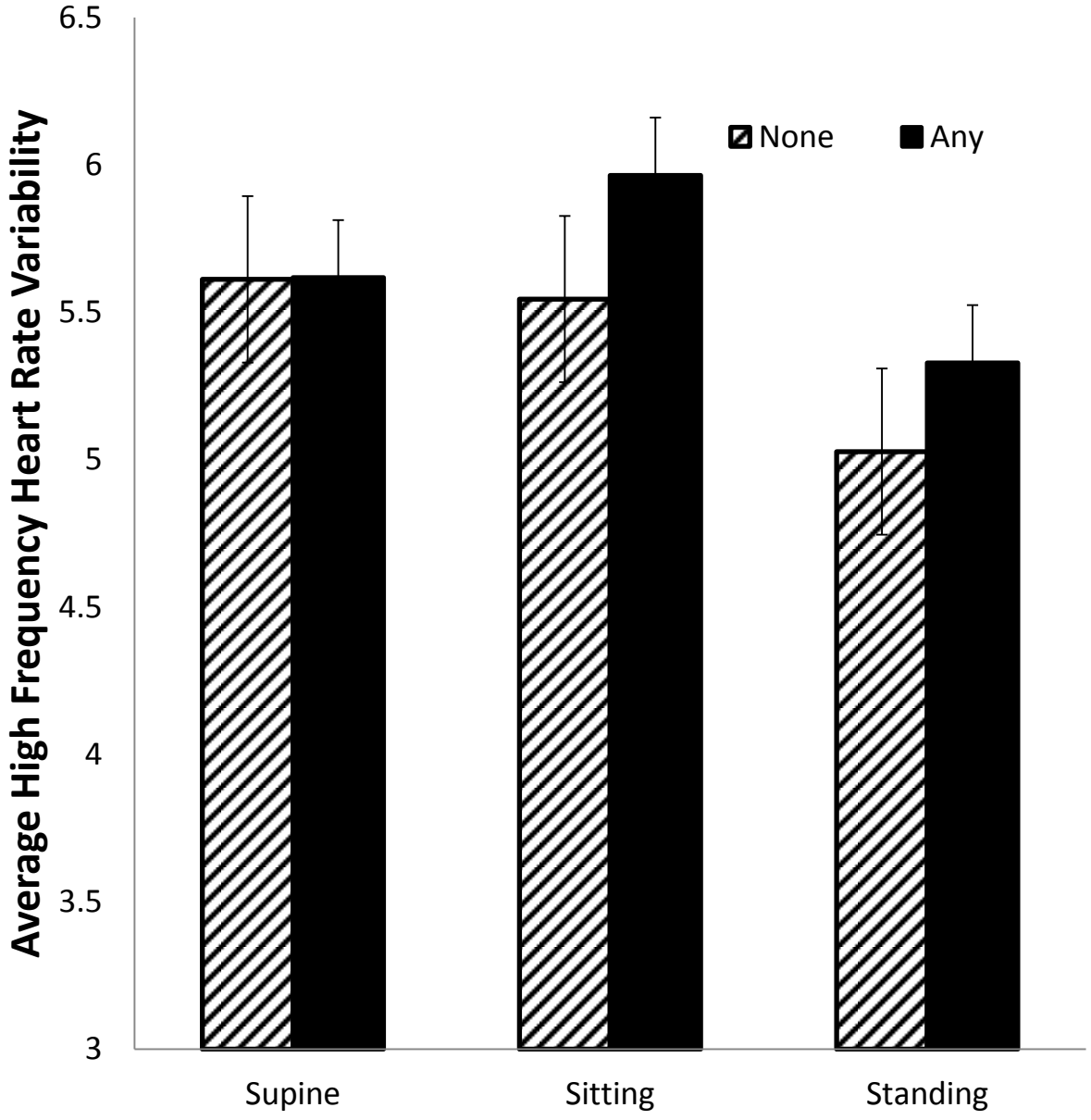


Figure 6. Average High Frequency HRV across Orthostatic Challenge Body Positions by Objective Hot Flash Group (No Hot Flashes vs. Any Hot Flashes).



v. sAA

As hypothesized, levels of sAA changed across the day, with a significant decrease in sAA from wake to thirty minutes after wake (β= 0.50, *SE*= 0.16, *p*= .002, *d*= 0.73), indicative of a significant sAA awakening response. This expected downward inflection in the levels of sAA 30-minutes after wake are expected and validate our measurements. Levels of sAA then increased between thirty minutes and three hours after wake *(*β= -0.80, *SE*= 0.16, *p*< .001, *d*= -1.16). Levels of sAA did not differ between the last three time points (*p's*> .23). Changes in sAA across the day by subjective HF group are shown in Figure 7. As predicted, in a MRM model examining the effects of subjective HFs on sAA there was no main effect of diary group on sAA across the day (β= 0.20, *SE*= 0.22, *p*= .37, *d*=-0.44). Furthermore, levels of sAA did not differ as a function of subjective HF group at any one time point (*p's*> .23).

When comparing objective HF groups, it was again observed that levels of sAA changed across the day. In addition, as predicted, across groups there was a significant decrease in sAA from wake to thirty minutes after wake (β= 0.50, *SE*= 0.16, *p*= .002, *d*= -0.18), again indicative of a sAA awakening response. Levels of sAA then increased between thirty minutes and three hours (180 min) after wake (β= -0.80, *SE*= 0.16, *p*< .0001, *d*= -0.58). As predicted, levels of sAA did not differ between groups at any of the last three time points (*p's*> .22). Contrary to predictions, when the data were compared between objective HF groups, there was no main effect of HF group on sAA across the day (β= 0.19, *SE*= 0.24, *p*= .42, *d*= -0.55). As expected, when examining differences at each time point, we observed a significant difference in levels of sAA at thirty minutes after wake (β= 0.71, *SE*= 0.32, *p*= .03, *d*= -0.73). However, the direction of the effect was contrary to predictions; women who had any objective HFs during the monitoring session had significantly higher sAA levels at 30 minutes than women who had no

objective HF. This analysis suggests a significantly attenuated sAA awakening response in women with objective HF compared to women with no objective HFs. Women with and without objective HFs did not have significantly different sAA levels at any other time point (*p's*>.13). Changes in sAA across the day by objective HF group are shown in Figure 8.

Figure 7. Natural log of Salivary Alpha-amylase across the Day by Subjective Hot Flash Group (Low Frequency vs. High Frequency).

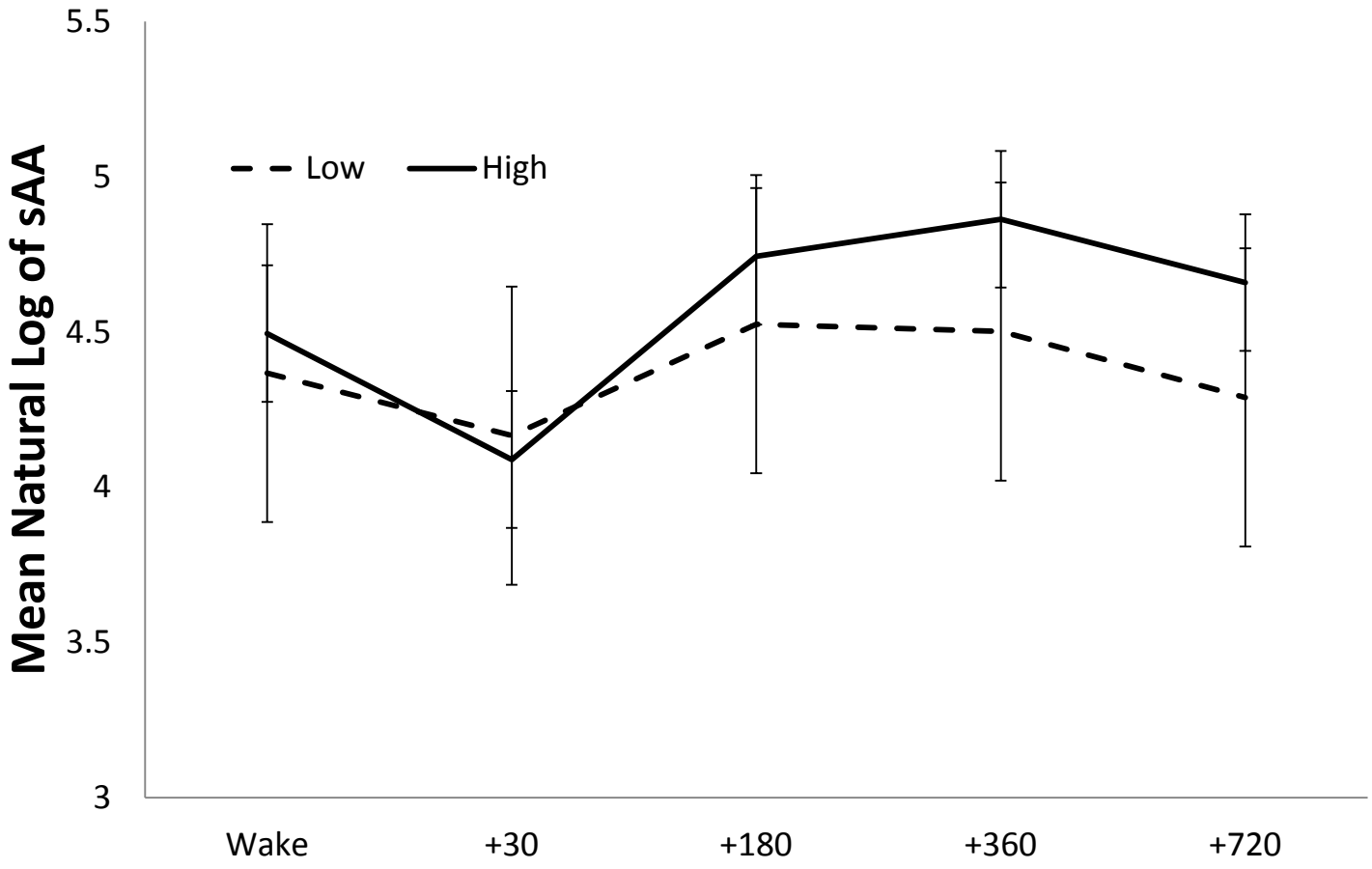
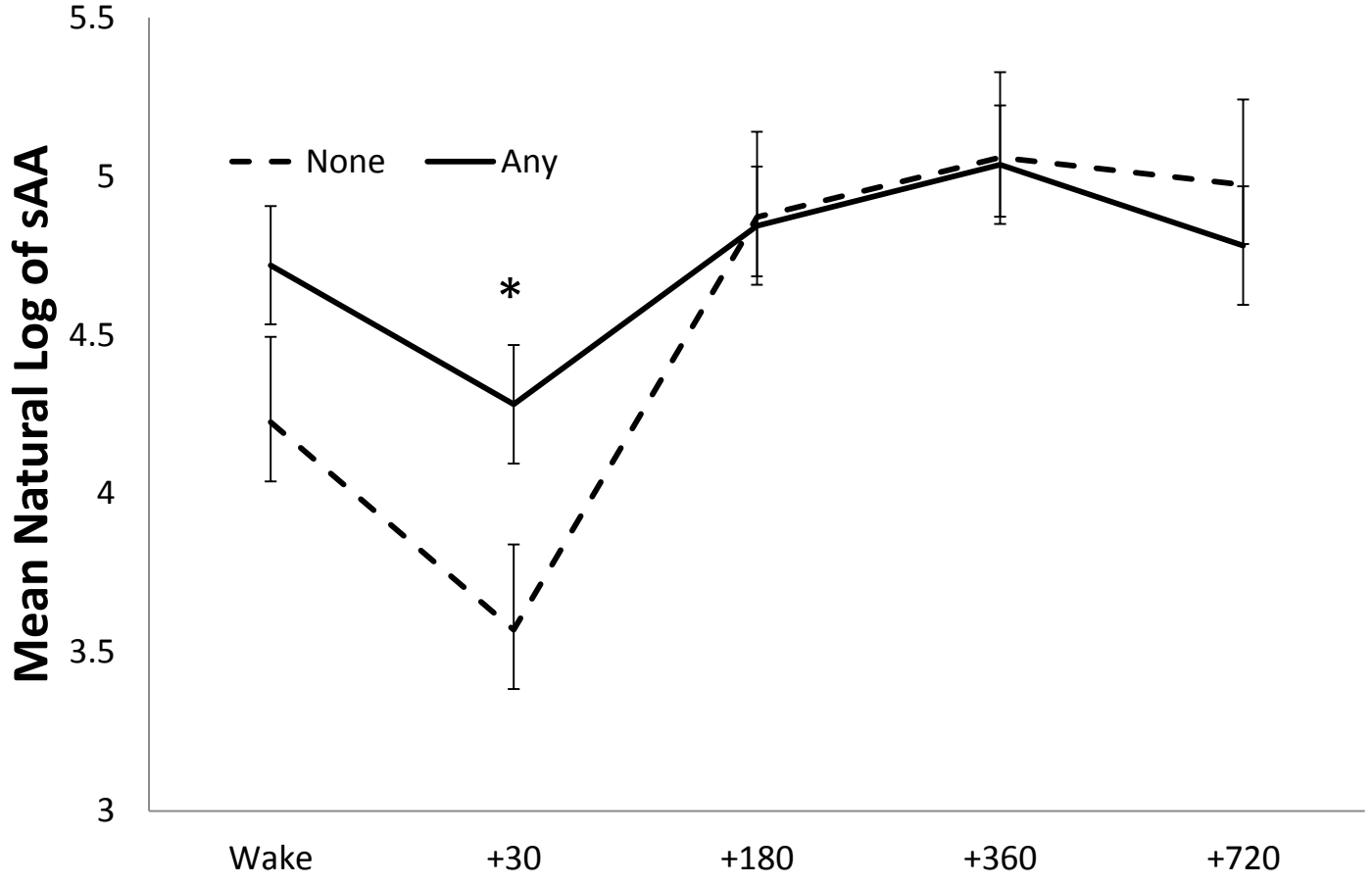


Figure 8. Natural log of Salivary Alpha-amylase across the Day by Objective Hot Flash Group (No Hot Flashes vs. Any Hot Flashes).



*p<0.05

b. Exploratory sAA Analysis

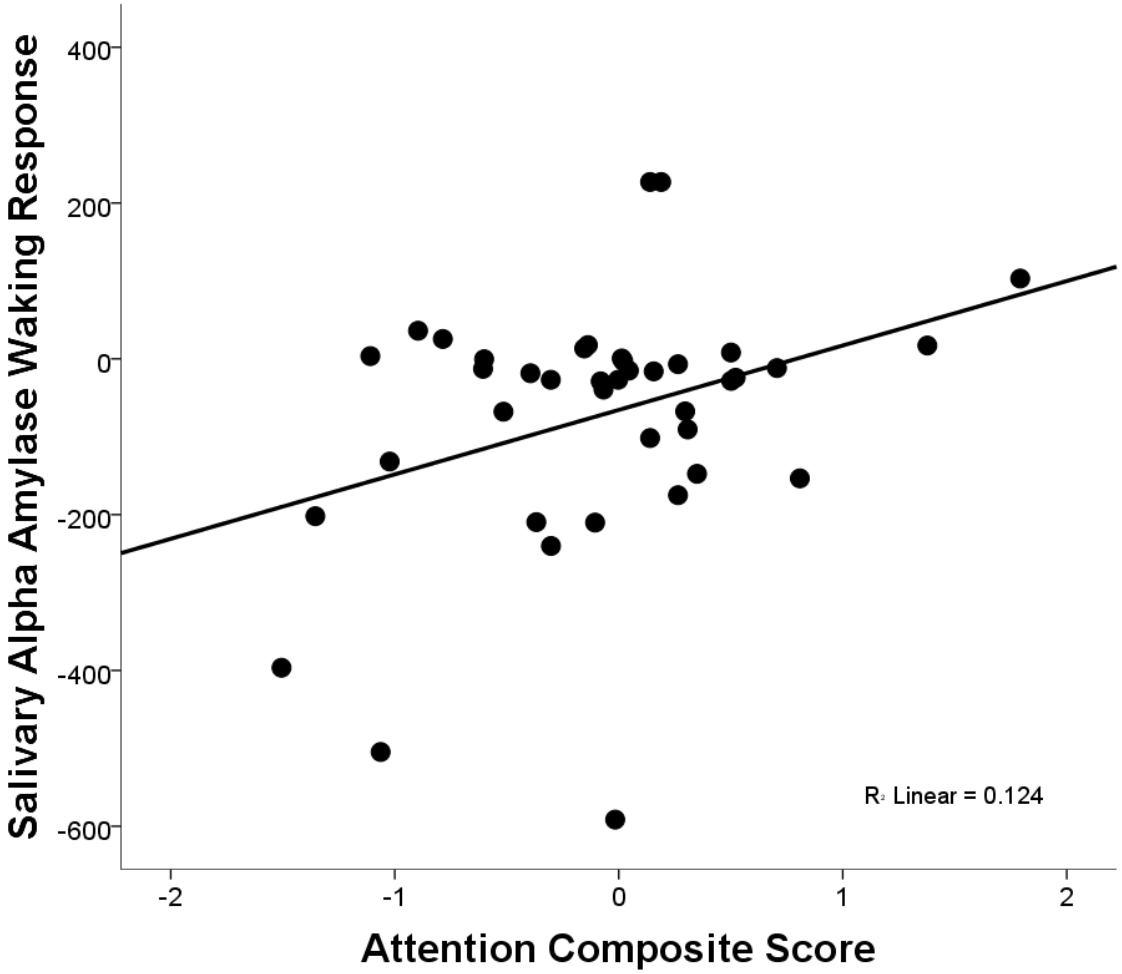
The previous analyses compared sAA in groups of women who differed in HF frequency but did not evaluate individual differences in sAA as a function of HF frequency (e.g., a dose response analyses). We therefore conducted a series of planned exploratory correlation and regression analyses to determine if individual differences in sAA outcomes – specifically AUC and awakening response - were associated with individual differences objective and subjective HF as well as cognitive (i.e., attention and memory domain scores) and psychological outcomes. Results based on Pearson correlational analyses showed that sAA awakening response was significantly and negatively correlated with frequency of objective ($r(38) = -.45$, $p = .004$), but not subjective ($p > .05$) HFs. Specifically, increasing numbers of objective HF were associated with lower sAA awakening responses. These results parallel our findings from the MRM models, and additionally suggest that there is a dose-dependent relationship between sAA awakening response and objective HF frequency.

Similarly, AUC sAA was significantly and positively related to objective ($r(38) = .35$, $p = .03$), but not subjective ($p > .05$) HFs. To further explore the observed relationship between sAA AUC and HFs (objective, subjective) a stepwise linear regression analyses was run. The total number of objective HFs was the only potential predictor that met threshold ($p \leq .10$) for entry into the regression equation with AUC of sAA. The total number of objective HFs was a significant predictor of AUC of sAA ($R^2 = .12$, $F(1, 39) = 5.19$, $p = .03$).

In order to determine if there were any relationships between sAA outcomes (AUC and awakening response), psychological outcomes and cognitive domain scores (i.e., memory and attention), another set of correlations were conducted. Potential predictors which met threshold ($p \leq .10$) for entry into the stepwise regression equation included: total score on the GCS, total

number of objective HFs and the attention domain score. Both the total number of objective HFs and the attention domain score were significantly associated the sAA awakening response (R^2 = .33, $F(1, 39)= 9.30$, p =.001). For attention, the direction of the effect was such that the greater the magnitude of the awakening response, the lower the score on the attention composite (β= 0.37, SE= 31.38, p= .009). This relationship can be seen in Figure 9. There was also a significant negative relationship between sAA awakening response and the total number of objective HFs (β= -0.46, SE= 2.78, p= .002). These data suggest that women who had frequent objectives HFs were more likely to have a blunted awakening response of sAA. Again, these results suggest that there is a dose-dependent relationship between objective HF frequency and sAA awakening response.

Figure 9. Salivary Alpha-amylase Awakening Response is positively associated with the Attention Domain Score.



VII. DISCUSSION

a) Summary.

The primary aim of this study was to investigate potential differences between ANS activity in women with and without frequent HFs by measuring HRV during an orthostatic challenge and sAA across the day. We predicted that objective HF would show a stronger relationship with ANS outcomes than subjective HF. Our first aim was to compare parasympathetic nervous system activity, as measured by high frequency HRV, between both women with low and high frequency subjective HFs, and between women with and without objective HF. Our prediction that objective, but not subjective HFs would be associated with decreased HRV was not supported. Our second aim was to compare sympathetic nervous system activity as measured by sAA across the day. We predicted that objective, but not subjective HFs would be associated with an exaggerated awakening response in sAA. That prediction was not supported. Instead, we observed a significant difference in levels of sAA at thirty minutes after wake but in a direction contrary to predictions. Specifically, women who had any objective HFs during the monitoring session had a significantly higher levels of sAA at 30 minutes after awakening compared to women with no HFs. In regression analyses, which examined HF frequency continuously rather than by group, both AUC of sAA and sAA awakening responses were significantly associated with the daily frequency of objective HF. This pattern of effects suggests that midlife women with objective HFs have an imbalance in ANS activity, with an increase towards sympathetic drive, as indexed by an increase in AUC with respect to ground (Gallicchio et al., 2010; Tuomikoski, Haapalahti, Sarna, et al., 2010; Tuomikoski, Haapalahti, Ylikorkala, et al., 2010).

b) Objective HF and ANS Outcomes

There is increasing evidence that measuring both objective and subjective HFs is important to understand the effect of HFs on women's health during the menopausal transition. There are distinct physiological and psychological symptom clusters which are differentially related to objective and subjective HFs. The advent of physiological monitoring of HFs has allowed our lab, and others, to demonstrate relationships between objective, but not subjective, HFs and cognition (Jamadar et al., 2014; Maki et al., 2008a) and cardiovascular risk factors (Thurston et al., 2011; Tuomikoski et al., 2009a; Tuomikoski, Mikkola, Tikkanen, & Ylikorkala). When only using self-reported HFs the relationship between cognition and HFs is not evident. Within our study population, this pattern of effects was demonstrated once again. Specifically, we found a dose/response relationship between AUC of sAA and objective HFs, and not subjective HFs, as well as an association between sAA awakening response and objective HF, but not subjective HF. These findings, in combination with previous research, underscore the importance of investigating both objective and subjective HFs.

c) sAA and HFs

Previous research studies have demonstrated a reliable time course of sAA across the day, with a downward inflection in sAA thirty minutes after awakening (Nater, Hoppmann, & Scott, 2013; Nater & Rohleder, 2009; Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007; Rohleder et al., 2004b; Strahler, Berndt, Kirschbaum, & Rohleder, 2010). We also found evidence of the previously reported pattern in sAA; however, in our sample women with any objective HFs showed less of a downward inflection in sAA 30 minutes after awakening. Furthermore, a higher frequency of objective HFs, when examined continuously, was associated with a blunted sAA awakening response. These data are suggestive of a dose-response relationship between the sAA

awakening response and objective HFs. Additionally, blunted sAA awakening response was related to both experiencing any objective HFs and better attention performance. Despite being a reliable effect, the mechanisms underlying the sAA are unknown and the clinical significance has yet to be determined; however the sAA awakening response appears to be unrelated to age (Nater et al., 2013). There is also evidence that individuals with generalized social anxiety disorder have a blunted awakening response in sAA (van Veen et al., 2008). Within our study population, anxiety symptoms were somewhat related to the sAA awakening response, but the effect did not reach significance (r = -0.26, p = .11); furthermore, anxiety symptoms were not related to the frequency of objective HFs (r = .09, p = .60).

Although the mechanism underlying the awakening response in sAA is still unknown, the cortisol awakening response is a well-established metric to investigate basal stress reactivity (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010; Clow, Thorn, Evans, & Hucklebridge, 2004; Schmidt-Reinwald et al., 1999). The diurnal rhythm of cortisol is opposite of sAA such that, cortisol reliably increases shortly after wake, and steadily decreases across the afternoon hours (Clow et al., 2010; Clow et al., 2004; Pruessner et al., 1997). The awakening response of cortisol is under the control of the hippocampus, and may be distinct from the normal rhythm of cortisol secretion across the day (Bruehl, Wolf, & Convit, 2009; Buchanan, Kern, Allen, Tranel, & Kirschbaum, 2004; Fries, Dettenborn, & Kirschbaum, 2009; Pruessner, Pruessner, Hellhammer, Bruce Pike, & Lupien, 2007; Schmidt-Reinwald et al., 1999; Wolf, Fujiwara, Luwinski, Kirschbaum, & Markowitsch, 2005). There is a possibility that the awakening response of sAA and the overall diurnal rhythm of sAA are also under the control of distinct mechanisms.

Previously, increased sympathetic activity, as measured by blood pressure, was reported in women with a history of self-reported HFs. To investigate whether individual differences in sympathetic drive across the day (and not just following awakening) were related to objective HF, we used a linear regression to evaluate the relationship between individual differences in the frequency of objective HFs and AUC of sAA. AUC with respect to ground is a measurement of total output, or exposure to sAA across the timeframe of monitoring (Fekedulegn et al., 2007; Pruessner et al., 2003). AUC is also used to evaluate dose/response relationships (Maes, Calabrese, & Meltzer, 1994). Here we found a dose-dependent positive relationship between objective, but not subjective HF, and increased basal sympathetic drive. Most (Gallicchio et al., 2010; Gerber, Sievert, Warren, Pickering, & Schwartz, 2007; Tuomikoski, Haapalahti, Ylikorkala, et al., 2010), but not all (Hautamaki et al., 2011), previous studies have suggested that HFs are associated with a shift in the autonomic balance towards increased sympathetic drive to the heart. To our knowledge this is the first investigation of the relationship between vasomotor symptoms and sympathetic nervous system activity as measured by sAA activity. Within our study sample, we found additional evidence for a relationship between sympathetic nervous system activity and HFs. Specifically, our results suggest a dose-dependent relationship between the frequency of objective HFs and AUC of sAA.

Our study used limited samples across the day, specifically at awakening and, 30 minutes, 180 minutes, 450 minutes and 720 minutes after awakening. Future research should explore the relationship between objective HFs and a more inclusive time course of sAA across the day. It would be informative to know if sympathetic activity was heightened across the entire morning, or only 30 minutes after awakening. Finally, more research should be conducted to determine if

the awakening response of sAA is both independent from the diurnal rhythm of sAA secretion and driven by the hippocampus.

d) HRV and HFs

Although we failed to see a difference in HRV by subjective or objective HF frequency, our data provide new insight in the relationship between objective HFs and HRV, and extend the interpretations of the previous investigations. Previous research has suggested that objective HFs are associated with withdrawal of vagal input to the heart (Freedman et al., 2011; Thurston et al., 2010, 2012). Initially, the most notable differentiation between previous studies was the use of objective or subjective HFs as the comparison group. Among such studies, only those which utilized objectively measured HFs found a negative relationship between HRV and vasomotor symptoms (Freedman et al., 2011; Thurston et al., 2010, 2012). In contrast, studies that measured subjective HFs failed to see an association between HFs and HRV (Hautamaki et al., 2012; Hautamaki et al., 2011; Hoikkala et al., 2010; Lantto et al., 2012).

Another factor that seemed to influence the relationship between HFs and HRV was the time course across which HRV was analyzed. Studies that utilized designs which focused only on HRV during the period immediately surrounding each objective HF event saw an acute effect of the HF on HRV, such that there is a transient withdrawal of the parasympathetic tone during objective HFs (Freedman et al., 2011; Hoikkala et al., 2010; Thurston et al., 2010, 2012). Furthermore, studies that investigated HRV across a longer time period (i.e. 24-hour period or during cardiovascular ANS testing), did not see any relationship between HRV and HFs (Hautamaki et al., 2012; Hautamaki et al., 2011; Hoikkala et al., 2010).

The one investigation, which had a design similar to ours, did not observe any differences in measures of HRV across an active orthostatic challenge in women with and without a history of

self-reported HFs (Hautamaki et al., 2012). Initially, we interpreted this as evidence that only objective HF would be related to decreases in HRV; however, our results paralleled their findings of no differences in HRV across a supine and standing orthostatic challenge in women with and without frequent subjective and objective HFs. Our investigation is the first to examine HRV in women with and without objective HFs across an orthostatic challenge and we found no differences. Overall, studies of HRV and HFs may suggest that HFs are associated with acute changes in the parasympathetic branch of the ANS at the time of the HF but are not associated with generalized state changes (i.e., changes not time-locked to an individual HF) in parasympathetic function. In this way, HFs might not affect overall daily (i.e., chronic) parasympathetic activity, but may transiently decrease parasympathetic output during each HF. Furthermore, our findings indicate that it is not possible to unmask alterations in parasympathetic tone in women with HF using a validated orthostatic challenge. Such differences might be evident only in the time period immediately surrounding a HF.

e) Psychological Outcomes, ANS activity and HFs

i) Mood Outcomes

Our data are consistent with previous research suggesting that women with frequent subjective HFs are more likely to have increases in subjective mood and sleep complaints (Fu, Matthews, & Thurston, 2014). Furthermore, these results complement a large body of previous research that has suggested patient reported and physiological HFs are related to distinct symptom clusters (Bromberger et al., 2007; Bromberger et al., 2010; Freeman, Sammel, Boorman, & Zhang, 2014; Freeman et al., 2009; Freeman et al., 2005; Freeman, Sammel, Lin, & Nelson, 2006; Fu et al., 2014). Specifically, our data suggest that women with frequent subjective HFs, but not frequent objective HFs, are more likely to have increased complaints of

sleep disturbances, anxiety and depressive symptoms. Our findings replicate previous studies suggesting that negative mood, subjective vasomotor symptoms and sleep complaints are interrelated (Shaver, 2009). Previous research has suggested that women with subjective HFs are more likely to develop a mood disorder (Cohen, Soares, Vitonis, Otto, & Harlow, 2006) and that HFs and mood disturbances are causally related (Freeman et al., 2009). Additionally, anxious women are more likely to overestimate their symptoms, and this effect is exacerbated when women had poor sleep quality the previous night (Fu et al., 2014). In clinical practice, it may be helpful to screen women for mood disruptions if their primary complaint is vasomotor symptoms.

ii) Cognitive Outcomes

Previous research has suggested sympathetic activity was positively related to attention and memory performance (Cahill & Alkire, 2003; Cahill & McGaugh, 1998; Eldar et al., 2013); however we did not see a relationship between memory performance and sAA. Exploratory analyses revealed that women with larger magnitude of an awakening response of sAA were performing significantly worse on the attention composite score. These data suggest that women with state elevated sympathetic activity perform better on measures of attention. An exaggerated awakening response in sAA would suggest decreased exposure to sympathetic arousal across the morning. One potential explanation that the preclinical literature has suggested is that epinephrine has an inverted-U dose response curve (Gold & Van Buskirk, 1976a, 1976b). Women who had an exaggerated decrease in sAA within the first thirty minutes after wake may have been pushed too low on the curve for optimal performance on attention tasks. While these data suggest a possible role of sAA awakening response in attention, these data should be

replicated in a larger sample, as there is the potential this relationship was seen due to type 1 error.

f) Study Limitations

The primary aim of this study was to investigate potential differences in ANS activity associated with both objective and subjective HFs. One main limitation of this cross-sectional design is that we cannot explore a causal relationship between HFs and our outcome measures (HRV and sAA) because we cannot determine temporal direction. Future research should aim to determine the time course of ANS alterations in relation to the development of HFs across the menopausal transition. Changes in ANS function should also be examined in the context of potential treatments for HFs. A second limitation of this study is the small sample size. All findings should be replicated in a larger sample size. One final limitation is that we were unable to measure flow rate of the saliva samples as they were collected outside of the laboratory. Some (Anderson et al., 1984; Asking & Gjorstrup, 1987), but not all (Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004a), previous studies have suggested that concentrations of sAA may be mediated by ANS control of salivary flow rate. One strength of this design was the collection of both subjective and objective measures of HFs in the same cohort of participants, which allowed for the comparison of the two measures on outcomes of interest, and which again underscored the importance of objectively measuring HFs.

g) Conclusions

The purpose of this study was to examine potential difference in ANS activity between women with and without frequent subjective and objective HFs. We found that women with objective HFs have a significantly attenuated awakening response in sAA. Furthermore, there was a dose-dependent association with the frequency of objective HFs predicting both AUC of

sAA and sAA awakening response. These data provide support that there is a state increase in sympathetic nervous system activity in women who have objective HFs. Additionally, our data provide new insight in the relationship between objective HFs and HRV. Previous research has suggested that objective HFs are associated with withdrawal of vagal input to the heart. Our data extend these findings to suggest that changes to the parasympathetic branch of the ANS associated with HFs may be transient. The decrease in parasympathetic activity may be only associated with each acute objective HF, not to a state decrease in parasympathetic tone, or reactive to an orthostatic challenge. We failed to find evidence to support ANS involvement in psychological outcomes between women with and without HFs; however, we found that sAA awakening response was independently associated with a composite measure of attention. These data provide interesting insight into the etiology of HFs, providing further support for the involvement of the ANS. Additional research is needed to replicate these findings in a larger cohort of women, specifically including a broader range of subjective HFs.

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AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
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Chicago, Illinois 60612-7227

Approval Notice
Continuing Review

January 22, 2014

Leah Rubin, PhD
Psychiatry
Center For Cognitive Medicine
912 S. Wood St., Rm. 827, M/C 913
Chicago, IL 60612-7327
Phone: (312) 355-5017 / Fax: (312) 413-8837

RE: Protocol # 2011-0273
"A Pilot Study of Mechanisms Underlying the Relationship between Hot Flashes and Memory Dysfunction"

Dear Dr. Rubin:

Please note that the research training ***will*** **expire** on **02/16/2014** for ***Antonia Savarese;*** on ***03/12/2014 for Dr. Mary Kapella*** and the research training ***will*** **expire** on **03/20/2014** for ***Erin Sundermann*.** Each of these individuals must respectively complete a minimum of two hours of continuing education in order to participate in the conduct of the research. You may refer them to the OPRS website, where continuing education offerings are available: http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/education/2-2-2/ce_requirements.shtml

Your Continuing Review was reviewed and approved by Members of IRB #3 by the Expedited review process on January 17, 2014. You may now continue your research.

Please note the following information about your approved research protocol:

Protocol Approval Period: January 17, 2014 - January 17, 2015
Approved Subject Enrollment #: 60
Additional Determinations for Research Involving Minors: These determinations have not been made for this study since it has not been approved for enrollment of minors.
Performance Sites: UIC
Sponsor: Chancellor's Discovery Fund for Multidisciplinary Research
PAF#: Not available
Grant/Contract No: Not available
Grant/Contract Title: A Pilot Study of Mechanisms Underlying the

Relationship between Hot Flashes and Memory Dysfunctions

Research Protocol(s):

a) A Pilot Study of Mechanisms Underlying the Relationship between Hot Flashes and Memory Dysfunction, Version #10; 10/21/2013

Recruitment Material(s):

a) Main Flyer: "Female Research Participants Needed!" (includes tear-off Version 3, 01/31/2012
b) "Post For Craig's List and UIC Announce", Version 3, 02/28/2012
c) Flyer "Are you postmenopausal?" (w/ tear-offs); Version #1; 08/06/2012
d) Telephone Screening Script - Cognition and Menopausal Symptoms; Version #4; 10/21/2013
e) "Initial Email Script"; Version #3; 10/21/2013
f) Patient Education: "Procedures/Diagnostic Tests; 24-hour urine collection" (as submitted to OPRS on 03/31/2011)

Informed Consent(s):

a) Consent: Cognition and Menopausal Symptoms-, Version #7, 03/20/2013
b) Alteration of Informed Consent granted under 45 CFR 46.116(d) for Telephone Screening Script

Your research continues to meet the criteria for expedited review as defined in 45 CFR 46.110(b)(1) under the following specific categories:

(2) Collection of blood samples by finger stick, heel stick, ear stick, or venipuncture as follows:
(a) from healthy, nonpregnant adults who weigh at least 110 pounds. For these subjects, the amounts drawn may not exceed 550 ml in an 8 week period and collection may not occur more frequently than 2 times per week; or
(b) from other adults and children, considering the age, weight, and health of the subjects, the collection procedure, the amount of blood to be collected, and the frequency with which it will be collected. For these subjects, the amount drawn may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period and collection may not occur more frequently than 2 times per week.,
(3) Prospective collection of biological specimens for research purposes by noninvasive means.
(4) Collection of data through noninvasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving X-rays or microwaves. Where medical devices are employed, they must be cleared/approved for marketing. (Studies intended to evaluate the safety and effectiveness of the medical device are not generally eligible for expedited review, including studies of cleared medical devices for new indications.)
(7) Research on individual or group characteristics or behavior (including but not limited to research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies.

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
01/14/2014	Continuing Review	Expedited	01/17/2014	Approved

Please remember to:

→ Use your **research protocol number** (2011-0273) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements on the enclosure,
"**UIC Investigator Responsibilities, Protection of Human Research Subjects**"
(http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/policies/0924.pdf)

Please note that the UIC IRB has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact OPRS at (312) 996-1711 or me at (312) 355-2939. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Jewell Hamilton, MSW
IRB Coordinator, IRB # 3
Office for the Protection of Research Subjects

Enclosure(s):

1. **Informed Consent Document(s):**
   a) Consent: Cognition and Menopausal Symptoms-, Version #7, 03/20/2013
2. **Recruiting Material(s):**
   a) Main Flyer: "Female Research Participants Needed!" (includes tear-off Version 3, 01/31/2012
   b) "Post For Craig's List and UIC Announce", Version 3, 02/28/2012
   c) Flyer "Are you postmenopausal?" (w/ tear-offs); Version #1; 08/06/2012
   d) Telephone Screening Script - Cognition and Menopausal Symptoms; Version #4; 10/21/2013
   e) "Initial Email Script"; Version #3; 10/21/2013
   f) Patient Education: "Procedures/Diagnostic Tests; 24-hour urine collection" (as submitted to OPRS on 03/31/2011)

cc: Anand Kumar, Psychiatry, M/C 912
Lauren S. Castro, CRC, 148 CSB, MC 596

CITED LITERATURE

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Zanchetti, A., Facchetti, R., Cesana, G. C., Modena, M. G., Pirrelli, A., Sega, R., & participants, S. (2005). Menopause-related blood pressure increase and its relationship to age and body mass index: the SIMONA epidemiological study. *J Hypertens, 23*(12), 2269-2276.

VITA
Lauren L. Drogos

EDUCATION

B.S. Psychology, University of Illinois, Urbana-Champaign, May 2005.
Thesis: Sex and Age Effects on the Basolateral Nucleus of the Amygdala
Advisor: Dr. Janice Juraska

M.A. Psychology, University of Illinois, Chicago, September 2009.
Thesis: Endogenous Oxytocin across the Menstrual Cycle and with Oral Contraceptive Use: Effects on Verbal Memory and Mood
Advisor: Dr. Pauline Maki

Ph.D. Psychology, University of Illinois, Chicago, August 2014.
Dissertation: Autonomic Nervous System Activity and Menopausal Symptoms
Advisor: Dr. Pauline Maki

RESEARCH EXPERIENCE

2005-2014 **Graduate Research Assistant. University of Illinois at Chicago.** Completion of subject recruitment, data collection and analysis including the following protocols:

- Effects of Black Cohosh and Red Clover on Cognitive Function in Menopausal Women
- Effect of Adjuvant Chemotherapy on Cognitive Function of Women with Early Stage of Breast Cancer
- Cognition, Brain Function, and Affect in Midlife HIV+ Women: The Influence of Menopause
- Effects of Oral Contraceptives on Memory
- Hot Flashes and Memory in Men undergoing Depot GnRH Analog Therapy for Prostate Cancer
- Effects of Estradiol and Soy on Menopausal Symptoms
- A Randomized, Placebo-Controlled Trial of Stellate Ganglion Block in the Treatment of Hot Flashes
- A Pilot Study of Mechanisms Underlying the Relationship between Hot Flashes and Memory Dysfunction

TEACHING EXPERIENCE

2007-2008 Graduate Teaching Assistant, Introduction to Psychology, Department of Psychology, University of Illinois at Chicago, Chicago, IL

2008-2009 Graduate Teaching Assistant, Physiological Psychology, Department of Psychology, University of Illinois at Chicago, Chicago, IL

2012 Graduate Teaching Assistant, Neuroanatomy, Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL

2010-2014 Guest Section Lecturer, Biology of the Brain, Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL

UNDERGRADUATE MENTEES

2007 – 2012 **Rhoda Jamadar, B.S.** Primary gradate mentor on a project ent"Hot Flashes and Memory in Men undergoing GnRH Analog Therapy for Prostate Cancer"

- 2009 Winner of Caterpillar and Kabbes undergraduate excellence funds
- 2010 Hodgkin and Huxley Neuroscience Award
-

2009 – 2013 **Lacey Wisslead, B.S.** Primary gradate mentor on a project entitled "Effects of Oral Contraceptive Use on Heart Rate Variability during Laboratory Induced Stress"

- 2011 Winner of Caterpillar and Kabbes undergraduate excellence funds
- 2011 Endocrine Society Summer Research Fellowship

HONORS AND AWARDS

2006 **North American Menopause Society New Investigator Award** This award recognizes the outstanding abstract submissions of four investigators who have achieved their degree within the past seven years.

2006 **Top Clinical Poster at the North American Menopause Society Conference** ($1000) for a project entitled Objectively Determined Hot Flashes: Evidence of Underreporting by Women and Negative Associations with Memory

2009 **Ob. Gyn. News Article** covering 2009 poster presentation at 20th annual meeting of the North American Menopause Society

2010 **Graduate College Student Travel Award** for poster presented at the 21st annual meeting of the North American Menopause Society.

2011 **Graduate College Student Travel Award** for poster presented at the 22nd annual meeting of the North American Menopause Society.

2011 **Graduate Student Council Travel Award** for poster presented at the 22nd annual meeting of the North American Menopause Society.

2011 **Top Abstract 2011 North American Menopause Society** Annual meeting in Washington, D.C. for a project entitled Circadian Rhythm of Hot Flashes in Symptomatic Postmenopausal Women and Men Undergoing GnRH Analog Therapy

2012 **Top Graduate Poster at UIC Student Research Forum** for a project entitled Objective Hot Flashes Negatively Related to Spatial Ability in Men with Prostate Cancer Undergoing Androgen Deprivation

2013 **Graduate College Student Travel Award** for poster presented at the 24th annual meeting of the North American Menopause Society.

2013 **Society for Women's Health Research (SWHR) What an "X" Makes Conference Top poster award** for project entitled A Comparison of Objective and Subjective Measures of Hot Flash Frequency in Midlife Women and Men Undergoing GnRH Analog Therapy

2013 **Provost's Award for Graduate Research** for a project entitled Effects of Sympathetic Autonomic Nervous System Function, Sleep and Menopausal Symptoms on Cognition

2014 **Graduate Student Council Travel Award** for poster presented at the 24th annual meeting of the North American Menopause Society.

2014 **Graduate Student Council Travel Award** for poster presented at the 24th annual meeting of the North American Menopause Society.

CONFERENCE PRESENTATIONS

International

1. **Lauren Drogos**, Leah H. Rubin, Kristen L. Mordecai, Sue C. Carter, Hossein Pournajafi-Nazarloo, Pauline M. Maki (2007) Effects of oxytocin on mood in premenopausal women: Investigations of menstrual cycle effects and oral contraceptive use. Poster presented at the 2008 annual meeting of the International Society of Psychoneuroendocrinology, Madison, WI.

2. Gould, F., Woodard, J.L., Rubin, L.H**.**, **Drogos, L**., Weber, K., Cohen, M., Martin, E., & Maki, P.M. (February 2007). Procedural memory function in HIV+ women. Annual Meeting of the International Neuropsychological Society. Portland, OR.

3. Sundermann, E.E., Rubin, L.H., Mordecai, K.L., Eatough, E., **Drogos, L.,** Fornelli, D., & Maki, P.M. (March 2010). Effects of menstrual cycle phase and stress on cognitive flexibility. 14th World Congress of Gynecological Endocrinology. Florence, Italy.

4. Rubin, L.H., Carter, S.C., **Drogos, L**., Pournajafi-Nazarloo, H., Savarese, A., Sweeney, J.A., Maki, P.M. (April, 2011). Sex-specific associations between peripheral oxytocin and positive emotion perception in schizophrenia. International Congress of Schizophrenia Research. Colorado Springs, CO.

5. Maki, P.M., Savarese, A., **Drogos, L.L.**, Rubin, L.H., Banuvar, S., Shulman, L.P., & Walega, D. (March 2012). Cognitive function in relation to hot flashes in women undergoing stellate ganglion block: baseline findings. 15th International Society of Gynecological Endocrinology. Florence, Italy.

National

6. M.J. Rubinow, **L.L. Drogos**, J.M. Juraska. Sex and age affect spine density and dendritic branching in the rat basolateral amygdala. Program No. 889.5. *2005 Abstract Viewer/Itinerary Planner.* Washington, DC: Society for Neuroscience, 2005.

7. **L.L. Drogos**, B.N. Fender, S. Geller, S. Banuvar , P.L. Shulman, P.M. Maki (2006). Objectively Determined Hot Flashes: Evidence of Underreporting by Women and Negative Associations with Memory. Poster submitted to the 17th annual meeting of the *North American Menopause Society*, Nashville, TN.

8. **Drogos, L.L.**, Rubin, L.H., & Maki, P.M. (October 10th, 2013) Consistency of Hot Flashes across a 72-hour Monitoring Session: Self-Reported and Objectively Recorded Hot Flashes. Poster presented at the 24th annual meeting of the *North American Menopause Society*, Dallas , TX

9. **L.L. Drogos**, M. Farag, S. Geller, L.P. Shulman, S. Banuvar, P.M. Maki (2007). Subjective Memory Complaints are Related to Objective Cognitive Performance in Symptomatic Postmenopausal Women. Poster submitted to the 2008 annual meeting of the North American Menopause Society, Dallas, TX.

10. **L.L. Drogos**, V. Meyer, L.F. Fogg, M.R. Smith, S. Geller, L.P. Shulman, S. Banuvar, P.M. Maki (2009). Circadian Rhythm of Hot Flashes in Highly Symptomatic Postmenopausal Women. Poster submitted to the 2009 annual meeting of the North American Menopause Society, San Diego, CA.

11. Rubin, L.H., Carter, C.S., **Drogos, L.,** Pournajafi-Nazarloo, H.P., Sweeney, & J.A., Maki, P.M. (May 2010) Endogenous oxytocin correlates with improved clinical symptoms in schizophrenia. Annual Meeting of the Society of Biological Psychiatry. New Orleans, LA.

12. Rubin, L.H., Carter, C.S., **Drogos, L.,** Pournajafi-Nazarloo, H.P., Sweeney, J.A., & Maki, P.M. (September 2010). Oxytocin is associated with positive emotion perception in women with and without schizophrenia. Sixth Annual Interdisciplinary Women's Health Research Symposium. Washington, DC.

13. Savarese, A., Rubin, L.H., Mordecai, K., Eatough, E., **Drogos, L.,** Sundermann, E., & Maki, P.M. (November 2010) Personality and the stress response in women. 40th Annual Meeting of the Society for Neuroscience (SFN). San Diego, CA.

14. Maki, P.M., Rubin, L.H., Mordecai, K.L., Savarese, A., Eatough, E., & **Drogos, L.** (December 2010). Cortisol responsivity to social stress varies across the menstrual cycle. ACNP. Miami Beach, FL.

15. P.M. Maki, R.J. Jamadar, L. R. Rubin, D. Fornelli, **L.L. Drogos**, S. Geller, L.P. Shulman, S. Banuvar, D. M. Little (2011) Patterns of Brain Activation and the Neural Effects of Objective Hot Flashes. Poster presented at 2011 Annual Meeting of the North American Menopause Society, Washington D.C.

16. Wisslead, L.B., **Drogos, L.,** Rubin, L.H., Savarese, A., Mordecai, K.L., & Maki, P.M. (June 2012). Effect of Oral Contraceptive Use on Heart Rate Variability during Laboratory-Induced Stress. Endocrine Society's 94th Meeting and Expo. Houston, T.X.

17. **Drogos, L.L,** Jamadar, R., Rubin, L.H., Geller, S., Shulman, L.P., Banuvar, S., Niederberger, C., Deane,L., & Maki, P.M. (July, 2013). A Comparison of Objective and Subjective Measures of Hot Flash Frequency in Midlife Women and Men Undergoing GnRH Analog Therapy. Poster presented at the Society for Women's Health Research's What a Difference an "X" Makes Conference in Washington, DC. (Note: Top poster prize awarded at conference for L.L. Drogos).

Regional

18. **L.L. Drogos**, Rubin, L.H., Mordecai, K.M., Carter, S.C., Pournajafi- Nazarloo, H., Maki, P.M. (2008). Effects of oxytocin on mood in premenopausal women: An investigation of menstrual cycle effects and oral contraceptive use. (2008) Annual meeting of the Chicago Chapter of the Society for Neuroscience, Chicago, IL.

19. **L. Drogos**, R. Jamadar, S. Geller, L.P. Shulman, S. Banuvar, D. Welega & P.M. Maki (March 29th, 2012) Objective Hot Flashes Negatively Related to Spatial Ability in Men with Prostate Cancer Undergoing Androgen Deprivation. Therapy. Chicago Society for Neuroscience Annual Meeting.

INVITED TALKS

International

1. **L. Drogos** (March, 2013). Inter-relationships between Autonomic Nervous System Activity, Menopausal Symptoms and Cognition. Special Seminar at the University of Toronto.

2. **L. Drogos** (February, 2014). Inter-relationships between Autonomic Nervous System Activity, Menopausal Symptoms and Cognition. Special Seminar at the University of Calgary.

National

3. Rubin, L.H., Carter, C.S., **Drogos, L.,** Pournajafi-Nazarloo, H.P., Sweeney, J.A., & Maki, P.M. (November 2010). Oxytocin is associated with positive emotion perception in women with and without schizophrenia. BIRCWH Scholars Meeting. Washington, DC.

4. **L. Drogos,** R. Jamadar, S. Geller, L.P. Shulman, S. Banuvar, D. Welega & P.M. Maki (September 23, 2011) Circadian Rhythm of Hot Flashes in Symptomatic Postmenopausal Women and Men Undergoing GnRH Analog Therapy. North American Menopausal Society 2011 Annual Meeting in Washington D.C.

PUBLICATIONS

1. **Drogos, L.L**, Geller, S., Maki, P.M. (2008) Soy and Cognition in the Aging Population. In R. Watson. Complementary and Alternative Medicine: Botanical Therapies to Promote Health in the Aged. Elsevier, Kidlington UK.

2. M.J. Rubinow, **L.L. Drogos**, J.M. Juraska. (2009). Age related dendritic hypertrophy and sexual dimorphism in rat basolateral amygdala. *Neurobiology of Aging*, 30(1), 137-46.

3. Maki, P.M., **Drogos L**, and Geller, S.G. (2009) Botanicals and cognitive function. In Watson R and Preedy V. Botanical Medicine in Clinical Practice. CABI publishing, Wallingford UK.

4. Maki, P. M., **Drogos, L. L**., Rubin, L. H., Banuvar, S., Shulman, L. P., & Geller, S. E. (2008). Objective hot flashes are negatively related to verbal memory performance in midlife women. Menopause, 15(5), 848-856.

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MANUSCRIPTS UNDER REVIEW

1. Jamadar, R., **Drogos, L.L.,** Rubin, L.H., Deane, L., Peace, D., Niederberger, C., & Maki, P.M. Objective vasomotor symptoms are associated with decreases in visuospatial function in men undergoing GnRH analog therapy for prostate cancer

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RESEARCH TECHNIQUES

1. Neuropsychological Testing
2. Collection and analysis of skin conductance data from an objective hot flash monitor (Biolog Model 3991x/2-HFI)
    a. Training multiple research labs on this method of data collection and analysis
3. Collection and analysis of heart rate variability data (Biolog Model 3991x/2-HFI)
    a. Data cleaning utilizing CardioEdit v 1.5 and CardioBatch
4. Collection and analysis of actigraphy sleep data (Actiwatch Score and Actiwatch 2, Minimiter)
5. Completing enzyme linked immunoassays; oxytocin, vasopressin, DHEA and cortisol.