VASOMOTOR SYMPTOMS AND NEGATIVE AFFECT: AN AMBULATORY ASSESSMENT OF MIDLIFE WOMEN

by

Carolyn Jo Gibson

BA, University of Virginia, 2002

MPH, Dartmouth College, 2008

MS, University of Pittsburgh, 2011

Submitted to the Graduate Faculty of the

Kenneth P. Dietrich School of Arts and Sciences in partial fulfillment

of the requirements for the degree of

PhD in Clinical and Biological/Health Psychology

University of Pittsburgh

2015

UNIVERSITY OF PITTSBURGH

DIETRICH SCHOOL OF ARTS AND SCIENCES

This dissertation was presented

by

Carolyn Gibson

It was defended on

November 11, 2014

and approved by

Rebecca C. Thurston, PhD, Associate Professor of Psychiatry, Epidemiology, Psychology, and Clinical and Translational Science

Stephen Manuck, PhD, Distinguished University Professor of Health Psychology and Behavioral Medicine

Anna Marsland, PhD, Associate Professor, Department of Psychology

Thomas Kamarck, PhD, Professor, Department of Psychology

Dissertation Advisor: Karen Matthews, PhD, Distinguished Professor of Psychiatry, Professor of Epidemiology and Psychology

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Carolyn Gibson, PhD, MPH

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Background: Hot flashes and night sweats, or vasomotor symptoms (VMS), are reported by an estimated 70-80% of women during the menopausal transition. Measures of negative affect are among the strongest and most consistent correlates of all aspects of VMS experience, though the mechanisms linking these factors are unclear. The current study aimed to examine the within day and day-to-day relationships between vasomotor symptoms and negative affect, and the potential role of sleep disturbance and cortisol dysregulation in these relationships, in a sample of women in midlife.

Methods: Fifty-three women (49% African American) who reported daily vasomotor symptoms were enrolled in an ambulatory study. For seven days, participants documented their mood state, VMS experience, sleep, and health behaviors multiple times a day using electronic diaries, and wore Actiwatches to capture additional data related to sleep parameters. Participants also provided morning and bedtime saliva samples for salivary cortisol collection over three days during the observed period. Hierarchical linear regression was used to examine relationships between VMS, negative affect, and related factors.

Results: Accounting for a number of health and demographic variables, women reported more negative affect on both the same day (β=1.46, $p<.001$ for VMS bother) and the day following (β=0.80, p=0.02 for night sweat severity, β=0.61, p=0.02 for night sweat bother) a more negative experience of vasomotor symptoms. A flatter diurnal cortisol slope was related to hot flash severity (β=0.09, p=0.03) and bother (β=0.10, $p<.01$) as well as negative affect (β=0.68, p=0.01), and partially explained the relationship between negative affect and VMS. Sleep disturbance did not appear to play a role in linking VMS to next day negative affect.

Conclusion: The subjective experience of VMS plays a key role in relationships between VMS, negative affect, and health-related factors on a daily basis. The findings of this study do not

support a small role of sleep disturbance in linking night sweat severity to next day negative affect, but suggest that further research is warranted to better understand the relationship between daily VMS experience, stress physiology, and negative affect.

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PREFACE

Support for this study was provided by funds from the American Psychological Association Dissertation Research Award, University of Pittsburgh Department of Psychology Diversity Committee Mini-Grant, University of Pittsburgh Department of Psychology A. David Lazovik Student Award, and the University of Pittsburgh Institute for Clinical Research Education.

1.0 INTRODUCTION

Hot flashes and night sweats, or vasomotor symptoms (VMS), are reported by an estimated 70-80% of women during the menopausal transition (Gold et al., 2006). The frequency, duration, severity, and bothersomeness of VMS are highly variable, and explanatory mechanisms for these differences in experience are only partially understood. Measures of negative affect are among the strongest and most consistent correlates of all aspects of VMS experience (Woods, Mitchell, & Landis, 2005), though the mechanisms linking these factors are unclear. Further, this relationship is typically explored in cross-sectional or longitudinal studies in which negative affect and VMS are retrospectively reported at one point in time, which contributes methodological concerns and provides no information about the daily experience of these variable symptoms. While the current literature has established that negative affect and VMS are strongly associated when measured at one point in time, limitations in study design do not allow for an understanding of the daily experience of these variable symptoms, their temporal associations, or what mechanisms might explain these associations.

A number of factors may explain the relationship between affect and VMS. Disrupted sleep in midlife is often attributed to VMS and posited to contribute to impaired next-day psychological functioning, as well as increased risk for depression over time. However, this relationship is not always seen in research, particularly when using objective measures of sleep or VMS (Joffe et al., 2009). Disrupted sleep may also contribute to dysregulation of diurnal

cortisol rhythms (Balbo, Leproult, & Van Cauter, 2010), which has been linked to depression and other measures of negative affect (Heaney, Phillips, & Carroll, 2010). In addition to this indirect pathway, cortisol dysregulation may link VMS and negative affect more directly. VMS, as a frequent, uncontrollable, often publicly embarrassing stressor, has been posited to increase cortisol levels as symptoms occur, contributing to cortisol dysregulation and changes in affect over time, particularly with frequent and/or severe VMS (Meldrum et al., 1984). Cortisol dysregulation has been researched in the context of negative affect, but not well-studied in relation to VMS.

This study aims to use ambulatory data collection to investigate the daily and day-to-day associations between negative affect and VMS, and to explore explanatory mechanisms linking these factors. The following pages review the common presentation and etiology of VMS, and the current literature on cross-sectional and temporal associations between negative affect and VMS. Potential explanatory mechanisms to be investigated are reviewed next, and the study design and analytic plan are then presented.

1.1 BACKGROUND

1.1.1 Vasomotor symptoms in midlife

Vasomotor symptoms (VMS), commonly referred to as hot flashes and night sweats, are experienced as temporary sensations of intense heat accompanied by sweating and flushing (Thurston et al., 2009). VMS are a common experience of the menopausal transition, reported by an estimated 70-80% of women. Prevalence increases with advanced menopausal stages, with

VMS most often occurring in the late perimenopause and early post-menopause (Gold et al., 2006). Despite the common occurrence of these symptoms, the pathophysiology leading to VMS is only partially understood. Current evidence best supports a thermoregulatory theory of VMS etiology.

Core body temperature follows a tightly controlled circadian rhythm, with integrated processing in the hypothalamus, body, and peripheral vascular operating to maintain a homeostatic state in response to changes in the external and internal environment. Sweating and blood flow are the primary mechanisms for regulating body temperature (Deecher & Dorries, 2007). VMS, characterized by sweating and peripheral vasodilation, are considered rapid and dramatic heat dissipation responses, responding to signals indicating an increase in core body temperature. Core body temperature may not be truly elevated prior to VMS, but VMS may result from a disruption or miscommunication in temperature signaling between the brain and peripheral vasculature. A common hypothesis is that the normal homeostatic thermoregulatory range controlled by the hypothalamus is altered during the menopausal transition, resulting in a narrowed thermoneutral zone. Within these tighter temperature constraints, even a small and typically insignificant elevation in core body temperature could trigger heat dissipation events such as VMS (Freedman, 2005). Another possibility is that the peripheral vasculature may have reduced responsivity with aging or menopause, contributing to delayed and altered responses of the vasculature to temperature signaling from the hypothalamus and body. Finally, hypothalamic neural functioning and neurochemical signaling related to thermoregulation (Deecher & Dorries, 2007), possibly including central serotonergic, noradrenergic, opioid, adrenal, and autonomic systems (Thurston, Christie, & Matthews, 2010), may be affected during this period.

Fluctuating gonadal hormone levels during the perimenopause and declining estradiol in the postmenopause are thought to play a key role in disrupting central thermoregulatory processing. Changing estrogen levels likely impact all of these possible routes, with regulatory effects on hypothalamic thermoregulation, effects on endothelial function in the vasculature, and influence on neurochemical release as well as neurotransmitter receptor gene expression and function for key neurotransmitters related to temperature signaling (Deecher & Dorries, 2007). The primary role of estrogens in the etiology of VMS is supported by the onset of VMS during the menopause and other periods of estrogen withdrawal, as well as the efficacy of exogenous estrogen administration in reducing or eliminating VMS. However, VMS are not experienced by all women experiencing either gradual or sudden estrogen withdrawal (Freedman, 2005). Further, the frequency, duration, severity (Woods et al., 2005), and bothersomeness (Thurston et al., 2008) of VMS are highly variable among women. The reasons for these individual differences in VMS are unclear, and cannot currently be explained by these hormonal or biological factors. A number of demographic and psychosocial risk factors seem to be more consistently and strongly related to all aspects of VMS experience. VMS are more common among women with lower socioeconomic status, smokers, and higher body mass index (BMI) (Thurston & Joffe, 2011), though associations between BMI and VMS occurrence may vary by age or menopausal stage (Thurston, Santoro, & Matthews, 2011). Measures of negative affect are a consistently found correlate of self-reported VMS experience (Woods et al., 2005).

1.1.2 Vasomotor symptoms and negative affect

Depressive and anxiety symptoms and disorders are often reported during the menopausal transition (Joyce T. Bromberger & Kravitz, 2011), and most investigations suggest that the risk

for depression in midlife is more common among women with VMS than those without VMS (Joffe et al., 2009). Vulnerability to mood symptoms during the perimenopause has been attributed to frequent and bothersome VMS (Woods et al., 2005). In a number of cross-sectional analyses of samples of women in midlife, self-reported VMS occurrence, frequency, severity, and bother have been associated with anxiety (Blümel et al., 2004; Cheng et al., 2008; Hunter, Gupta, Papitsch-Clark, & Sturdee, 2009; Li, Yu, Ma, Sun, & Yang, 2008; Seritan et al., 2010; Thurston et al., 2008, 2009), depressive symptoms (Blümel et al., 2004; Brown, Gallicchio, Flaws, & Tracy, 2009; Cheng et al., 2008; Hunter, Gupta, Papitsch-Clark, & Sturdee, 2009; Joffe et al., 2002; Li, Yu, Ma, Sun, & Yang, 2008; Seritan et al., 2010; Thurston et al., 2008, 2009), psychological distress (Joyce T. Bromberger et al., 2003; J. T. Bromberger et al., 2001; Ishizuka, Kudo, & Tango, 2008), and negative affect (Collins & Landgren, 1994; Thurston et al., 2008). Different aspects of VMS experience may be more or less related to mood symptoms; negative affect is a significant correlate of self-reported VMS bother even after adjusting for frequency (Thurston et al., 2008). The results of some studies suggest that VMS may be more strongly related to mood symptoms than to clinically significant mood disorders (Bosworth et al., 2001; Elavsky & McAuley, 2009; Oztürk, Eraslan, Mete, & Ozşener, 2006; Schmidt, Haq, & Rubinow, 2004), and that associations between negative affect and VMS may be strongest in perimenopausal women compared to symptomatic pre- or postemenopausal women (Cheng et al., 2008; Joffe et al., 2002; Seritan et al., 2010). Overall, most studies support an association between measures of negative affect and aspects of VMS experience, though much remains to be clarified about this association.

The nature of the association between negative affect and VMS are not well understood, largely due to limitations in the typical measurement of VMS in the literature. In research, VMS

is usually assessed with retrospective recall, queried as the occurrence of symptoms in the past one, two, or four weeks. This methodology introduces issues of inaccuracy, as reliability may be reduced after even brief delays in symptom recall. In one investigation, recall of VMS frequency at the end of the day resulted in underestimated VMS reporting compared to prospective self-reported and physiologically detected VMS throughout that same day (Fu, Matthews, & Thurston, 2014). Retrospective recall of somatic symptoms is also known to be biased by mood at the time of reporting, further contributing to reporting inaccuracies as well as potentially creating or inflating the relationship seen between affect and self-reported VMS.

The influence of negative affect on VMS perception and reporting is highlighted by studies comparing self-report of VMS with objective VMS, typically physiologically detected with sternal skin conductance. The correlation between reported VMS and objective VMS occurrence is not high (Sievert, 2013), and associations between self-report VMS and negative affect are not always seen with objective VMS. When both self-report and physiological VMS are measured, self-reported VMS not corroborated by physiological evidence seem to be more common among women with high levels of anxiety (Thurston, Blumenthal, Babyak, & Sherwood, 2005; Thurston, Hernandez, Del Rio, & De La Torre, 2011) and depressive symptoms (Thurston et al., 2005). With these issues in mind, future research on associations between VMS and negative affect should limit retrospective recall methods, account for trait-like characteristics related to negative affectivity, and recognize the potential role of negative affect in influencing perception and reporting of self-reported VMS.

1.1.3 Temporal associations between vasomotor symptoms and negative affect

An additional limitation of the current literature is an inability to establish the directionality of the association between VMS and negative affect. Cross-sectional methods support a relationship between VMS and negative affect when both are measured at one point in time, but do not allow for examination of the temporal associations between these factors. The commonly seen association between self-reported VMS and negative affect may be due to negative affect contributing to VMS reporting, or VMS leading to negative affect. Few studies have specifically tested these pathways. Negative affect leading to VMS on a year-to-year basis has been explored in four large epidemiological samples of women in midlife. In the Penn Ovarian Aging Study (POAS), an observational cohort study of over 400 women in midlife, both previous year anxiety and an increase in anxiety scores from the previous year predicted the initial onset of VMS, measured as any VMS in the previous month, during the menopausal transition (Freeman et al., 2005). In additional analyses of the POAS, the onset of depressive symptoms was more likely to precede the onset of any VMS among participants who had neither at baseline and developed both over the 10-year observed period (Freeman, Sammel, & Lin, 2009). Depressive symptoms at baseline predicted the development of VMS over the following four years of observation (Freeman et al., 2001). Depressive symptoms at baseline also predicted the onset of any self-reported menopausal symptoms, including but not limited to VMS, occurring at any time over ten years of observation in a large community sample in France (Sabia, Fournier, Mesrine, Boutron-Ruault, & Clavel-Chapelon, 2008), and self-reported bothersome VMS in the past two weeks over nine years among the 438 participants in the Melbourne Women's Midlife Health Project (Guthrie, Dennerstein, Taffe, Lehert, & Burger, 2005). Depressive and anxiety symptoms at baseline predicted both annually reported VMS occurrence and reporting VMS on

six or more days in the previous two weeks annually in the Study of Women's Health across the Nation (Gold et al., 2006). The alternate pathway of VMS leading to negative affect over time was also investigated in the Melbourne Women's Midlife Health Project, which found that depressive symptoms in the final study visit were predicted from the number of bothersome menopausal symptoms, including but not limited to VMS, at baseline ten years previously (Guthrie et al., 2005).

While epidemiological studies can explore the relationship between mood symptoms and disorders and VMS onset over the course of years, they provide no information about the within-day and day-to-day relationship between these variable factors among symptomatic women. Annually collected data is also limited by retrospective report of VMS, which may be inaccurate and/or biased by mood at the time of reporting. In contrast to the summary measures of negative affect and VMS collected in epidemiological studies, ambulatory and daily diary data can be utilized to investigate the relatively acute effects of negative affect on VMS, and vice versa, with limited retrospective recall bias. However, very few published studies have explored temporal associations between negative affect and VMS in this fashion.

Ambulatory measurement of self-reported and objectively measured VMS has supported a role of anxiety in predicting both types of VMS. However, while a number of factors were associated with objective VMS, only anxiety predicted VMS reporting in this sample (Thurston, Hernandez, et al., 2011). When both VMS and affect were measured in an ambulatory fashion, Thurston et al. (Thurston et al., 2005) found that self-reported VMS without corroborating physiological evidence were more likely to be reported in the minutes following increased frustration and decreased feelings of control. In contrast, physiologically identified VMS were less likely to follow increases in negative affect, and more likely to follow increases in positive

affect (Thurston et al., 2005). The alternate pathway, self-reported VMS leading to negative affect, is supported by the limited data available from two published diary studies investigating this relationship on a day-to-day basis. Burleson et al. (Burleson, Todd, & Trevathan, 2010) collected daily diary ratings of self-reported VMS and mood items from 55 healthy women in midlife for 36 weeks. Negative affect was measured as a mean composite score of daily ratings of mood symptoms (anxious, depressed, distressed, nervous, hostile, ashamed, guilty, and irritable), while VMS was categorized as any or no occurrence of VMS during each 24-hour period. VMS predicted higher next-day negative affect, but only in women who did not have high levels of depressive symptoms, measured with the Center for Epidemiological Studies for Depression (CESD) scale, at baseline (Burleson et al., 2010). While the findings supported the theory that VMS precedes negative affect over short periods of time, the effect of VMS on acute mood states was only seen in women with low stable measures of negative affect.

Gibson et al. (Gibson, Thurston, Bromberger, Kamarck, & Matthews, 2011) investigated the daily and day-to-day relationship between negative affect and VMS using data from the Daily Hormone Study, a subsample of women in the menopausal transition from the Study of Women's Health Across the Nation. In this multiethnic sample of 625 women, participants completed a daily diary each night before bed for approximately 30 days. Negative affect was measured as a mean composite variable of mood ratings (mood swings, feelings easily hurt, irritable, difficulty concentrating, forgetful, anxious, and blue/down), dichotomized to indicate the presence or absence of negative mood each day. As in Burleson et al. (2010), VMS was reported as any or no occurrence of VMS during each 24-hour period. In this analysis, higher average negative affect over the month of daily diary collection was associated with an increased risk of reporting VMS over the month, and higher negative affect was associated with reporting

VMS within each 24-hour period. On a day-to-day basis, VMS was associated with next day negative affect, while no association was seen between negative affect and next day VMS (Gibson et al., 2011).

In summary, negative affect as a trigger of self-reported VMS that do not meet objective criteria was supported by Thurston et al. (Thurston et al., 2005). Burleson et al. (Burleson et al., 2010) and Gibson et al. (Gibson et al., 2011) support daily relationship of VMS leading to negative affect, but both were limited by simplistic measures of both negative affect and VMS, with global negative and positive affect determined from ad hoc measurement of mood retrospectively reported at the end of the day, and VMS limited to a retrospective report whether or not any VMS occurred each day. No published study has adequately examined the day-to-day relationship between aspects of VMS experience, including frequency, severity, and bother of VMS, and independent aspects of negative affect. Improved measurement of prospectively reported depressive and anxiety symptoms is warranted to better assess these relationships. Replication and improvement upon existing methods would lend further support to this temporal association, while also adding to our ability to investigate potential mechanisms linking VMS and negative affect (figure 1).

1.2 MECHANISMS LINKING VASOMOTOR SYMPTOMS AND NEGATIVE AFFECT

Associations have been seen between VMS and negative affect on the same and subsequent day (figure 2). A number of factors may explain the temporal relationship between VMS and negative affect. On a day-to-day basis, VMS is thought to disturb sleep, with sleep problems in

turn contributing to next day negative affect (figure 3). VMS-related sleep disturbance may also contribute to biological disruption associated with negative affect, while VMS itself may serve as a stressor causing acute stress responses resulting in overall higher stress hormone exposure (figure 4) and, with frequent or severe VMS exposure, dysregulation of stress hormone rhythms, also leading to increased negative affect (figure 5).

1.2.1 VMS-related sleep disturbance contributing to next day negative affect

The prevalence of sleep problems increases with aging for both men and women, with a marked increase seen among women compared to men at midlife (Kravitz et al., 2011). At that time, an estimated 30-60% of peri- and postmenopausal women report sleep disturbance (Kravitz et al., 2008). Sleep problems are most common among women in midlife who also report VMS, and sleep difficulty is consistently associated with self-reported VMS in SWAN and other samples (Blümel et al., 2004; Kravitz et al., 2008, 2011). Disturbed sleep is known to be associated with mood disorders and symptoms, and contributes to impaired next-day psychological functioning (Balbo et al., 2010; Kumari et al., 2009). The domino hypothesis posits that VMS disturbs sleep, leading to next-day psychological impairment and negative affect (figure 3). The contribution of self-reported VMS to perceived sleep disturbance among women in midlife has been supported in a number of cross-sectional studies, and disturbed sleep is known to impact psychological functioning and next-day mood (Joffe et al., 2009). With frequent disturbance due to VMS, this cycle of chronic sleep disruption contributing to impaired daytime functioning and well-being could lead to the development of clinically significant depressive symptoms (Joffe et al., 2009). The directionality of this pathway has been supported by daily diary studies, in which self-reported trouble sleeping in the intervening night played a small but significant role in explaining

the relationship between VMS and next-day negative affect (Burleson et al., 2010; Gibson et al., 2011). However, as VMS and sleep in these studies was only measured as retrospective self-reported single items indicating any occurrence of VMS, and the presence or absence of any trouble sleeping (Gibson et al., 2011) or sleep problems (Burleson et al., 2010) in the previous 24 hours, additional research with improved measurement of key factors is needed to better elucidate this relationship.

A significant contribution of VMS to sleep disruption and poor sleep quality is a necessary assumption of the domino hypothesis, but the relationship between sleep difficulties in the late perimenopause and postmenopause and self-reported VMS seen in cross-sectional research may instead reflect VMS effects on symptom perception and reporting. More bothersome self-reported overnight VMS are associated with sleep problems (Thurston et al., 2008) and reporting feeling less rested upon awakening (Thurston, Santoro, & Matthews, 2012). When physiological data and morning self-reports are compared, women reporting poorer sleep have been shown to overestimate overnight VMS the following morning (Fu et al., 2014). Further, an association between VMS and sleep is not always seen when sleep or VMS are assessed with objective measures rather than self-report (Thurston, Santoro, & Matthews, 2012). Women with frequent nocturnal arousals may attribute awakenings to VMS, regardless of whether the sleep disturbance was truly related to VMS occurrence (Thurston, 2006); women with VMS may report poor sleep, regardless of whether sleep disturbance seems to truly occur. No difference between total sleep time, awakenings, and brief arousals measured by EEG in women with and without skin conductance-measured VMS was seen in one study of 31 women in midlife; further, sleep arousals occurring within two minutes of a flash in symptomatic women were no more likely to occur after VMS than before (Freedman, 2004). In another study of 41

women with VMS, self-reported overnight VMS were not significantly associated with overnight VMS physiologically detected with skin conductance, and self-reported sleep complaints, depression, and anxiety were associated only with the self-reported VMS, but not with the physiologically detected VMS (Thurston, Blumenthal, Babyak, & Sherwood, 2006). In a subsample of 52 women from the Study of Women's Health across the Nation, while more self-reported VMS recalled upon awakening are associated with lower sleep efficiency and higher WASO as measured by actigraphy, physiologically measured VMS over the same night was not related to those sleep parameters (Thurston et al., 2012). Overall, these findings suggest that while women report sleep disturbance due to VMS, it is not clear that VMS is actually disturbing sleep in symptomatic women, or contributing to mood symptoms through this pathway.

Alternatively, objective measurement of sleep may not capture differences in nocturnal arousal that explain disparities between objective and subjective reports of sleep disturbance and poor sleep quality. While menopausal status or VMS frequency did not predict differences in polysomnography measures of sleep disturbance, including total sleep time and percent of time in each sleep stage, from 370 pre-, peri-, and postmenopausal participants in the SWAN Sleep Study, EEG data suggests that women in late perimenopause and postmenopause have a higher beta arousal level, commonly elevated in insomnia, during sleep. This may explain the subjective reporting of poorer quality and more disturbed sleep, despite no differences seen in PSG measures, predicted by both menopausal stage and self-reported VMS frequency in this sample (Campbell et al., 2011). Other studies have shown that patterns of sleep disturbance may differ over the course of the night, which may not be seen with traditional analytic approaches to objective sleep measurement. In a small sample of 36 women, Freedman et al. (Freedman & Roehrs, 2006) found that polysomnography-measured brief sleep arousals and awakenings were

more common in women with VMS than those without during the first half of the night, with VMS detected by skin conductance typically preceding arousal. This was not seen in the second half of the night, when REM sleep is more common and may be suppressing thermoregulatory response and subsequent VMS in symptomatic women (Freedman, 2006). Additionally, the subjective experience of VMS may play a role in the discrepancies found between self-reported and objectively measured VMS and sleep disturbance. Self-reported VMS severity was related to actigraphy-derived measures of sleep disturbance in a sample of 217 healthy postmenopausal women (Ensrud et al., 2009). Bothersome VMS, though not self-reported VMS occurrence or frequency, were associated with self-reported sleep disturbance in a sample of 623 women in midlife (Xu et al., 2012).

While evidence is mixed, and clouded by a number of methodological differences, ample evidence suggests that sleep may be affected by VMS perceived to be severe or bothersome, and that sleep disturbance contributes both acutely and cumulatively to negative affect. Further study is needed to clarify the relationship between self-reported VMS and objective measures of sleep disturbance, and whether VMS contributes to negative affect through detrimental effects on sleep parameters. Data collection over repeated days with self-reported and objectively measured sleep parameters and improved VMS reporting, including assessment of VMS bother and severity, is needed to adequately examine the relationship between subjectively reported VMS and sleep, as well as the potential role of sleep disturbance in explaining the relationship between VMS and negative affect on a day-to-day basis.

1.2.2 VMS-related cortisol dysregulation contributing to negative affect

Night sweats and hot flashes are often considered interchangeably, with reported values of each combined or hot flashes considered a proxy for night sweats. However, differences between night sweats and hot flashes, and differences in their associations with related factors such as negative affect, are not clear. While night sweats may disturb sleep, contributing to next day negative affect, hot flashes during the day may contribute to negative affect through differential pathways. One potential pathway is through hypothalamic pituitary adrenal (HPA) axis dysregulation, which is commonly studied in relation to depression and mood symptoms. If VMS contributes to stress-related cortisol overexposure and dysregulation (figure 4), this could be an additional pathway through which VMS contributes to negative affect (figure 5).

HPA axis dysregulation is often assessed in research by measuring urinary, plasma, or salivary cortisol. More recently, using hair samples to assay overall cortisol exposure over the past several months have also been utilized to assess HPA axis dysregulation and correlated health risks (Manenschijn et al., 2013). Cortisol typically follows a pattern in which levels peak in the early morning and decline steadily across the day, creating a diurnal slope. Levels also increase in response to stressors, particularly those involving socio-evaluative threat (Dickerson, Mycek, & Zaldivar, 2008). Alterations in these expected patterns are thought to indicate dysregulation, and have been associated with mood symptoms and disorders (Heaney et al., 2010) as well as sleep problems (Kumari et al., 2009). Depressive pathophysiology may result from persistent neurobiological changes related to hypercortisolemia (Palazidou, 2012), while disrupted sleep in turn impacts HPA axis activity, including increased cortisol stress reactivity (Hori et al., 2011), higher next-day cortisol levels (Buckley & Schatzberg, 2005, p. 200), and increased corticotropin releasing hormone and cortisol release nocturnal awakening, such as those possibly related to

VMS (Balbo et al., 2010). While a number of studies have linked parameters related to HPA axis dysregulation, most consistently diurnal slope (Heaney et al., 2010) with negative affect and sleep, a relationship between cortisol measures and VMS have been frequently suggested but not yet established.

HPA axis dysregulation may contribute to the etiology of VMS, as the HPA and hypothalamic pituitary ovarian (HPO) axis are tightly integrated (Woods, Carr, Tao, Taylor, & Mitchell, 2006), and menopause-related changes in gonadal hormone cyclity are likely to influence HPA function and feedback sensitivity, with the fluctuating and declining levels of estrogen in the peri- and postmenopause leading to a decreased regulation and increased expression of HPA axis activity. Age- and menopause-related increases in HPA axis activity may in turn further suppress HPO, affecting gonadal hormone secretion and hormonal influence on thermoregulation (Neal-Perry, Nejat, & Dicken, 2010). While dysregulation of the integration of these axes with aging and menopause is likely, these pathways are difficult to examine and disentangle in observational research. It has also been established that whatever the cause, changes in gonadal hormones and their potential influence on thermoregulation does not explain all of the variability in the occurrence or experience of VMS (Freedman, 2005). Alternatively, it has been posited that VMS may instead contribute to HPA axis dysregulation.

As an unpredictable and often socially embarrassing stressor (Smith, Mann, Mirza, & Hunter, 2011), VMS may trigger stress-related cortisol release and higher overall cortisol exposure across the day (Meldrum et al., 1984) (figure 4). VMS, particularly VMS reported as severe or bothersome, could therefore result in higher overall cortisol exposure and dysregulation of diurnal cortisol rhythms (Woods et al., 2006), in turn associated with increased negative affect. Data from several studies of very small samples suggest that mean serum cortisol

concentration increases 15 minutes after VMS, supporting an acute stress response to VMS among symptomatic women (Cignarelli et al., 1989; Genazzani et al., 1984; Meldrum et al., 1984). In a subsample of the Seattle Midlife Women's Health Study, overnight urinary cortisol levels were found to increase during the late perimenopause, and this increase was predicted by VMS severity (Woods et al., 2006). However, in another analysis of the same sample spanning the entire menopausal transition, VMS the same day or day following overnight urinary cortisol collection were slightly associated with lower cortisol levels (Woods, Mitchell, & Smith-Dijulio, 2009). In a study of 85 healthy, early postmenopausal women, higher scores on the Greene Climacteric Scale, a questionnaire quantifying mood and somatic symptoms related to the menopausal transition, was significantly associated with higher 24-hour urinary cortisol levels. The VMS subscale alone was not associated with cortisol levels, though with only two questions may not be a reliable measure of VMS experience (Cagnacci et al., 2011). In a study using salivary diurnal cortisol in 130 midlife women, no association was seen between self-reported VMS occurrence in the previous two weeks and diurnal cortisol patterns from one day of observation (Sievert, 2012). Finally, in a study using applied relaxation to treat VMS, both VMS frequency and salivary cortisol was lower among women who received the applied relaxation intervention (n=33) compared to controls (n=27) three months following the end of the intervention (Lindh-Åstrand & Nedstrand, 2013).

Though mixed, current findings overall generally support an association between increased cortisol exposure and VMS, potentially as a result of stress-related cortisol increase subsequent to VMS. However, interpretability of this small literature is limited by methodological concerns, including the small sample sizes (Cignarelli et al., 1989; Genazzani et al., 1984; Meldrum et al., 1984), use of overnight (Woods et al., 2006, 2009) and 24 hour urinary

cortisol (Cagnacci et al., 2011) to characterize typical cortisol exposure, and limited or retrospective measurement of VMS (Cagnacci et al., 2011; Sievert, 2012). Diurnal salivary cortisol sampling has long been recognized as the best measure of cortisol exposure (Kirschbaum & Hellhammer, 1989), and hair cortisol sampling has recently become recognized as a clinically useful and valid measure of longer-term cortisol exposure (Russell, Koren, Rieder, & Van Uum, 2012). To date, no published data has used these techniques to examine relationships between cortisol and prospectively reported VMS. Further, HPA axis dysregulation as part of a mechanistic pathway linking VMS and negative affect in a sample with measurement of cortisol, VMS, and negative affect has not yet been examined. Additional research is needed to establish the relationship between self-reported VMS and cortisol, and whether VMS may contribute to negative affect through changes in cortisol exposure. The complementary use of mulitiple days of salivary diurnal cortisol collection and hair cortisol analysis, related with improved VMS reporting, including assessment of VMS bother and severity, and improved measurement of negative affect, is needed to adequately examine the relationship between self-reported VMS and cortisol. These methodological improvements will also help inform the potential role of cortisol in explaining the relationship between VMS and negative affect on an overall and day-to-day basis.

1.3 SUMMARY

A relationship between VMS and negative affect is consistently seen in the literature, though the relationship between variable aspects of both symptom and mood experience in daily life have not been well-established. Though there is some evidence to suggest that VMS contribute to

negative affect, the mechanisms that underlie these associations are not well understood. Sleep problems are commonly reported among women in midlife, and often attributed to VMS. Sleep disturbance as a result of VMS may impair next-day psychological functioning and, over time, contribute to depression risk. However, while a role of VMS in interrupting sleep is often seen in cross-sectional studies with self-reported sleep and VMS, the results are less consistent when sleep and/or VMS are assessed with objective measures. The relationship between objective sleep measures, negative affect, and VMS has not been assessed in an ambulatory study where temporal associations between all can be assessed over time.

Additionally, disturbed sleep may contribute to dysregulation of biological mechanisms linked to negative affect, including HPA axis functioning and cortisol exposure. The experience of VMS as a chronic, unpredictable, socially relevant stressor may cause frequent stress cortisol responses to VMS, further contributing to cortisol dysregulation and providing another link between VMS and negative affect. It is therefore proposed that VMS will predict same day and next-day negative affect, with sleep disturbance playing a role between severe and bothersome night sweats and next day negative affect, and diurnal cortisol exposure influenced by both sleep disruption and severe VMS, and contributing to the association between VMS and same-day and overall negative affect. Though complex and bidirectional associations between VMS, sleep, cortisol, and negative affect are likely, the unidirectional pathways presented here were chosen as those most supported by the current literature, best integrating both sleep and cortisol as mechanisms linking VMS and negative affect, and testable by the proposed study.

For my dissertation study, I aimed to address the limitations of the current literature by using ambulatory methods to fully characterize and relate daily VMS and mood experiences of women across a typical week. I used objective methods to assess the role of sleep disturbance,

diurnal cortisol dysregulation, and longer-term cortisol exposure as mediators that may better explain the relationship between VMS and negative affect. Detailed assessment of daily VMS symptom experience, measurement of both depressive and anxiety symptoms, and stringent assessment of potential factors linking these common aspects of the menopausal transition was utilized to improve our understanding of the acute and cumulative contributions of VMS to aspects of negative affect among symptomatic women in midlife.

1.4 SPECIFIC AIMS AND HYPOTHESES

1.4.1 Specific Aim 1: Examine the contribution of VMS to within-day and next-day negative affect.

Hypothesis: VMS and negative affect will be associated on the same day, and VMS will predict next day negative affect (figure 1). These associations will be strongest with severe or bothersome VMS, consistent with the literature and the greater impact of severe and bothersome VMS on the health behaviors and physiological parameters proposed to link VMS and affect.

1.4.2 Specific Aim 2: Examine sleep as a mediator of the association between VMS and next-day negative affect.

Hypothesis: Actigraphy-determined sleep efficiency and wake after sleep onset will partially explain the relationship between VMS and next day negative affect (figure 2). Severe and/or

bothersome VMS in particular may disrupt sleep and contribute to arousal, awakening, and poorer sleep quality, impairing next-day psychological functioning.

1.4.3 Specific Aim 3: Examine cortisol as a correlate of VMS, negative affect, and sleep (a), and as a mediator of the association between VMS and negative affect (b).

Hypothesis: VMS, along with sleep and negative affect, will be associated with a flatter diurnal cortisol slope (figure 3) and higher overall hair cortisol exposure. VMS, particularly severe VMS, may contribute acutely to cortisol stress reactivity and, with frequent occurrence, diurnal cortisol dysregulation contributing to long-term cortisol overexposure. Sleep disruption is thought to contribute to increased cortisol secretion, autonomic activation, and corticotropin-releasing hormone release, contributing to circadian cortisol dysregulation, while neurobiological changes related to stress-related HPA axis overactivation and cortisol overexposure contributes to the development of depressive symptoms and disorders. Given these expected associations and pathways, cortisol exposure will also partially mediate the relationship between VMS and same day and overall negative affect (figure 4).

2.0 METHODS

2.1 DESCRIPTION

Participants were enrolled in a weeklong ambulatory study focused on VMS and negative affect. Following a telephone screen to determine eligibility and gather basic information, they completed an office visit consisting of informed consent and questionnaires. They were then sent home with handheld electronic diaries (Palm, Inc., Santa Clara, CA) equipped with pre-installed diary forms (Satellite Forms, Thacker Network Technologies, Inc.), an Actigraph, equipment to collect six samples of salivary cortisol, and written instructions for all study materials. During the ambulatory portion of the study protocol, they completed self-initiated diary entries upon wakening and before bed, and signaled diary entries at four additional timepoints throughout each day. They also completed salivary cortisol collection upon awakening and at bedtime on three consecutive weekdays during the data collection period. Upon completion, participants returned all equipment to the office in a prescheduled visit, where they also received compensation and were debriefed on the study. Participants were paid $50 for participation, split between completion of the laboratory portion ($10), ambulatory monitoring ($30), and cortisol collection ($10). Salivary cortisol collection days were scheduled for each individual such that they occurred on three consecutive weekdays, with efforts made to ensure

that VMS, mood, and sleep data collection occurred on at least one day/night prior to and following salivary cortisol collection.

2.2 SAMPLE

Fifty-three women from the community were enrolled in this study. Recruitment efforts included advertisements in the UPMC Extra, flyers in public spaces around the city and doctors' offices, and the University of Pittsburgh Clinical and Translational Science Institute's Research Participant Registry. Eligible participants were peri- or postmenopausal women who self-reported daily VMS. Women reporting current (within the past 3 months) use of oral contraceptives, hormone therapy, tamoxifen, aromatase inhibitors, SSRIs, SNRIs, lithium, antipsychotic medication, prescription sleep aids, corticosteroids, and any diagnosed primary sleep disorders were excluded from participation due to the known effects of these treatments and conditions on VMS, mood, sleep, and/or systemic cortisol levels.

2.3 MEASUREMENT

2.3.1 Telephone screen

Potential participants completed a telephone screen to determine eligibility. In addition to information about the inclusion/exclusion criteria, the screen queried demographic and menopause-related information with known associations with both VMS and negative affect,

including birth date and current age, race/ethnicity, highest level of educational attainment, self-rated health ("In general, would you say your health is excellent, very good, good, fair or poor?"), menopausal status based on self-reported bleeding patterns (early perimenopausal (bleeding in the last 3 months with some change in cycle regularity in the last 12 months), late perimenopausal (bleeding >3 months ago but within the last 12 months), postmenopausal (no bleeding in the last 12 months), smoking status (current yes/no, past yes/no), age, and vasomotor symptom experience (past two weeks frequency, typical severity (mild: sensation of heat without sweating; moderate: sensation of heat with sweating, able to continue activity; severe: sensation of heat with sweating, causing cessation of activity) (Guttuso, DiGrazio, & Reddy, 2012), typical bother (not at all, very little, moderately, a lot); lifetime duration) (Thurston et al., 2008). Participants were also be asked about current over the counter and prescription medication use, including oral contraceptives and hormone therapies, which may influence menopausal symptoms and diurnal cortisol patterns. The telephone script and all data captured during screening were entered through RedCap, a password-protected, encrypted, secured database administered by the University of Pittsburgh.

2.3.2 Office visit

Participants came to the research office for a scheduled 1-2 hour visit, during which time they reviewed and signed IRB-approved informed consent documents, received instructions on study equipment, and completed the following measures and tasks:

2.3.2.1 Questionnaires

Participants completed computer-based questionnaires using RedCap to assess a number of state and trait measures of psychological factors and health behaviors. Relatively stable traits related to anxiety were measured with the Anxiety Sensitivity Index (Taylor et al., 2007) and the Spielberger Trait Anxiety Scale (Spielberger, Gorsuch, R.L., Lushene, R., Vagg, P.R., & Jacobs, G.A., 1983). The Center for Epidemiological Studies Depression Scale (Radloff, 1977) was completed to assess depressive symptoms over the previous two weeks. The Mini-IPIP (Donnellan, Oswald, Baird, & Lucas, 2006), a 20-item short form of the 50-item International Personality Item Pool-Five-Factor Model measure (Goldberg, 1999), was used to assess personality measures consistent with the Big Five. Neuroticism was the trait personality measure of primary interest, given known associations between neuroticism and somatic symptom reporting (Feldman, Cohen, Doyle, Skoner, & Gwaltney, 1999) and a more stressful perception of the menopausal transition (Elavsky & McAuley, 2009). These longer-term measures were used to compare reported negative affect during the observed period to "typical" affectivity for each participant, and to test for moderation of relationships of transient mood states and VMS by more stable negative affect. Attitudes Toward Aging and Menopause (Sommer et al., 1999) were assessed, given known associations between these attitudes and both VMS and depressive symptoms among women in midlife (Ayers, Forshaw, & Hunter, 2010). Typical self-reported sleep was assessed using the Pittsburgh Sleep Quality Index (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989), in order to compare self-reported sleep measures to those objectively measured by actigraphy during the study. Typical physical activity, a health behavior that may influence both mood and VMS, was assessed with the International Physical Activity Questionnaire Short

Form (Craig et al., 2003). Finally, the Hot Flash Related Daily Interference Scale (Carpenter, 2001) was completed as an additional measure of the self-reported typical burden of VMS. Completion of these questionnaires took approximately 20-45 minutes, with most variability in duration due to the participants' varying levels of familiarity and comfort with using a computer.

2.3.2.2 Physical measures

BMI and waist circumference were obtained during the initial study visit, due to known associations between these measures and both negative affect and VMS. Trained staff measured participants' height and weight using a laboratory stadiometer (BMI was calculated from weight (kg) and height $(m)^2$), and waist circumference using a laboratory measuring tape. Waist circumference was measured in centimeters with a measuring tape placed around the participant at the narrowest part of the torso.

2.3.2.3 Hair cortisol

A sample of hair for cortisol analysis was obtained by trained study staff, cut from close to the scalp. Due to the increased likelihood of participant concern or discomfort about providing this sample, participants were given the option to refuse this portion of the protocol without withdrawing from the study. Providing a hair sample for cortisol analysis was not included in the criteria for receiving full payment for study participation.

2.3.3 Ambulatory monitoring

Following the laboratory visit, participants completed seven days of ambulatory monitoring.

2.3.3.1 Electronic daily diaries

Participants were given handheld Palm Pilots (Palm, Inc., Santa Clara, CA) with pre-installed diary forms (Satellite Forms, Thacker Network Technologies, Inc.) that they completed upon wakening, before bed, and at four additional times over the course of each day upon signaling from pre-programmed alarms (Appendix B). During the office visit, participants were trained on how to use the diary. While participants were reminded of the importance of responding to the alarm as soon after it sounds as possible, they were shown how to delay response when in situations that make responding immediately dangerous or difficult.

(a) Signalled within-day entries

Participants were signaled by their electronic diaries to complete a diary entry four times evenly spaced throughout the day, at 11 am, 2 pm, 5 pm, and 8 pm. While it was expected that sleep and wake times would be significantly variable between participants, and possibly within participants over the course of the observed period, these times were selected to maximize adherence (consistent signal times throughout the week, during times unlikely to coincide with meals or other obligations) and comparability between participants. Within-day entries briefly collected information on VMS and affect.

(i) Vasomotor symptom reporting

Participants were prompted to report the number of VMS experienced since the last report, as well as the peak severity and peak bother of VMS during that time. VMS severity categorization (1/mild: sensation of heat without sweating; 2/moderate: sensation of heat with sweating, able to continue activity; 3/severe: sensation of heat with sweating, causing cessation of activity) followed the FDA guidelines for defining VMS severity (Guttuso et al., 2012), while categorization of VMS bother (1/not at all, 2/very little, 3/moderately, 4/a lot) is consistent with measures in the literature (Thurston et al., 2008). Participants were also asked to report the approximate time of their last VMS.

(ii) Mood reporting

Participants were prompted to report the extent to which they were currently experiencing symptoms of positive or negative affect and anxiety, using prompts from the PANAS-X (Negative Affect Scale (afraid, scared, nervous, jittery, irritable, hostile, guilty, ashamed, upset, distressed), Positive Affect Scale (active, alert, attentive, determined, enthusiastic, excited, inspired, interested, proud, strong), and Sadness Subscale (sad, blue, downhearted, alone, lonely) (Watson & Clark, 1994). In order to enable a comparison of negative affect in this study from that of Gibson et al.(Gibson et al., 2011), additional ad hoc items (moody, sensitive, and forgetful) were also included in the response forms. All individual items were rated on a likert scale (1: very slightly or not at all, 2: a little, 3: moderately, 4: quite a bit, 5: extremely), consistent with recommended use of the PANAS (Watson & Clark, 1994) and typical use in ecological momentary assessment (Steptoe, Gibson, Hamer, & Wardle, 2007).

(iii) Contextual factors

To aid in interpreting mood states at the time of reporting, signaled diaries also queried participants about their external and interpersonal environment. These questions were, "Are you alone?" (yes/no) and "Where are you?" (1/work, 2/home, 3/in public). Each diary entry took approximately 5-7 minutes to complete.

(b) Self-initiated morning entry

Morning diary entries were completed upon awakening, designed to capture information on what time participants woke up, what time they got into bed, what time they think they fell asleep the night before, the prior night's sleep quality (including trouble sleeping, yes/no, for comparability with Gibson et al. (Gibson et al., 2011) and Burleson et al. (Burleson et al., 2010)), and the number, severity, and bother of any vasomotor symptoms experienced overnight.

(c) Self-initiated end-of-day entry

Bedtime diary entries were completed each night before sleep, designed to capture information on the time they went to bed, their mood at the time of entry, vasomotor symptoms (frequency, severity, bother) since the last entry, exercise (yes/no, time of day), alcohol use (yes/no, time of day of last use), tobacco use (yes/no, time of day of last use), and any naps (number, approximate time of day of all naps) experienced over the course of the day. Affect and VMS questions were equivalent to those collected throughout the day, so that this data could be included as a fifth timepoint in the day.

2.3.3.2 Actigraphy

Sleep was also assessed using actigraphy, with participants wearing Actiwatches throughout the observed period. Target sleep parameters included sleep duration, sleep efficiency, and wake after sleep onset. Actigraphy has been well-validated for the measurement of these parameters (Martin & Hakim, 2011), which relate to the sleep disruption thought to characterize the negative impact of VMS on sleep. Participants were also asked to press the event marker button on their Actiwatch each time they experienced vasomotor symptoms.

2.3.3.3 Salivary cortisol collection

At the office visit, participants were given six Salivette collection devices (Sarstedt, Numbrecht, Germany), cotton/polyester swabs in individual tubes that are commonly used for salivary cortisol collection, and instructed to collect six samples over three consecutive days. Participants were instructed to conduct salivary cortisol collection upon awakening and at bedtime (Adam & Kumari, 2009; Lederbogen et al., 2010). Participants were encouraged to keep the saliva kit next to their bed along with a pen to record collection times on the sample tubes (Halpern, Whitsel, Wagner, & Harris, 2012). Participants were instructed that "wake" time for the initial collection should mean, "When you open your eyes and are aware of being awake for the day, but before your feet touch the ground." Actigraphy wake times were compared to the time of data collection indicated for the initial sample collection to determine accuracy (Adam & Kumari, 2009). Participants were instructed on the timeline and procedure for collection during their laboratory visit. Participants were instructed to refrain from eating, smoking, or consuming alcohol for one hour prior to collection, and to chew on the swabs for at least 30 seconds before

transferring it back to the tube, which they then marked with their initials, date, and time of collection, and placed in their home refrigerators until their scheduled date to return supplies to the laboratory. In order to maintain stability of samples, salivary cortisol can be kept at 4–8 °C in household refrigerators for up to 7 days, and stored at 5 °C for up to 3 months or at -20 -80 °C for up to 12 months (Inder, Dimeski, & Russell, 2012).

2.4 DATA REDUCTION AND MANAGEMENT

2.4.1 Actigraphy

An Actiwatch-16 (Philips Respironics, Inc.) was worn on participants' non-dominant wrist continuously for 7 days and nights, with data stored in 1-minute epochs for the duration of the observed period. Sleep time was determined by participants' diary-reported time to bed and time awake, verified by representative changes in actigraphy. Validated software algorithms (Actiware Version 5.04) was used to estimate sleep efficiency and wake after sleep onset within the designated sleep intervals. Nightly values for each participant and an average value for each participant across the 7 nights of observation was also calculated and maintained in the dataset for analysis. Sleep efficiency, or the percentage of time in bed that is spent sleeping, was calculated as time spent asleep/time in bed × 100. Wake after sleep onset (WASO) was calculated as the total minutes of wakefulness between diary-reported sleep onset and final wake time. Awakenings were defined as a total activity count greater than a sensitivity threshold of 40.

2.4.2 Cortisol

Salivary and hair cortisol samples were sent to Dresden Lab Services in Dresden, Germany for processing, under the direction of Clemens Kirschbaum, PhD. Distributions of salivary cortisol data were examined for normality and outliers, and log-transformed to normalize the data. Assayed salivary cortisol values below 0.3 nmol/L and above 60 nmol/L were excluded from analyses. Diurnal slope was calculated from each participant's waking salivary cortisol value subtracted from their bedtime salivary cortisol value, adjusted by time between reported collection times for each participant, on each collection day. Analyses with cortisol slope were further adjusted by reported wake time for that morning. Both a daily value and an average value for each participant, calculated as the mean of their three collection day values, was categorized in the dataset. Two samples in each collection day were determined to be a reliable measure of diurnal slope, based on high correlations between diurnal slope calculated with just two values (waking and bedtime) and diurnal slopes calculated from four values (waking, +4 hours, +9 hours, and bedtime) seen on multiple days in a previous sample ($r=.97$-$.98$, $p<.05$; Karissa Miller, personal communication). Bedtime rather than a fixed point (ie, +16 hours after awakening) in the day was selected to maximize comparability between participants, based on expected inter-individual variability in typical day-length. Based on the same sample, it was expected that the measurements between days would be significantly, though not highly, correlated ($r=.39$-$.46$, $p<.05$; Karissa Miller, personal communication). Finally, one value, believed to estimate the overall cortisol exposure over approximately the previous 3 months (Stalder & Kirschbaum, 2012), was assessed for each participant from hair cortisol assays.

2.5 STATISTICAL ANALYSIS

The projected recruitment goal was set at 50 participants. Power analysis (Optimal Design software for nested models) suggested that n=46 at level 3 would provide 80% power with α=.05, moderate standardized effect size of .5, σ2=.53, 4+ observations at level 1 (number of within-day observations), and 7 observations at level 2 (number of days). Standardized effect size was derived from moderate to large effect sizes seen in the published literature and analysis of SWAN FLASHES diary data when looking at the differences in negative affect, anxiety, and depressive symptom scores by VMS reporting, VMS frequency, and VMS bother. The σ2 was derived from the level 1 variance seen in SWAN DHS, with VMS predicting next day negative affect.

Analyses were conducted with SPSS v. 20 and HLM v.7.01. Any outliers were identified and addressed, the distribution of study variables was examined, and variables were categorized as needed. Descriptive analysis was conducted to evaluate means and frequencies of all study variables. Within-day observations, with observations nested within days, and days nested within participants, were examined with three-level hierarchical linear models (HLM). Intercepts-only models were run to determine the variance in each level present for each outcome, and to verify that random intercepts were appropriate for these models. Associations between predictors and outcomes were assessed with random-coefficients models. Covariates, selected based on known associations with VMS and affect, were entered into models in a stepwise fashion. Base models were adjusted only by time of day of symptom reporting (morning, afternoon, evening), followed by demographic variables in model 2 (age, menopausal status, race/ethnicity, educational attainment), and finally by health-related factors (BMI or WC, physical activity, self-rated health, alcohol use, tobacco use) in model 3. For all variables,

measures for each day of observation as well as aggregate measures across the observed period were calculated and retained in the dataset. Though key variables are identified by their categorical terms for parsimony in the presentation of statistical analysis equations, each aspect of VMS, negative affect, and sleep defined below were examined in independent analyses.

3.0 RESULTS

3.1 CHARACTERISTICS OF SAMPLE AT BASELINE

A total of 53 participants completed in the study. In order to obtain that sample, 135 women were screened, 62 participants were determined eligible and enrolled, 7 enrolled participants failed to come to their scheduled appointments and were not successfully contacted to reschedule, and 2 enrolled participants decided to withdraw from the study prior to their scheduled appointment. Forty-four participants provided at least one day of morning and bedtime saliva samples that provided assays within normal limits to calculate diurnal slopes and daily cortisol exposure. Thirty-seven women provided hair samples for hair cortisol sampling. Those who did not provide hair samples were more likely to be African American, and non-participation in this aspect of data collection was generally due to having hair that was too short to provide a sufficient sample (3 centimeters required). Participants completed 5-9 (mean, 7.77) days of observation, with 1-14 (mean, 5.34) diary entries entered on each day of observation. Forty-seven participants completed at least 4 diary entries on 6 or more days of observation.

Participants were generally postmenopausal and overweight. Though not by design, the sample was half Caucasian and half African-American. Participants reported relatively positive attitudes toward aging and menopause (mean: 2.37 out of 3, SD: 0.42), and personality measure scores indicating a general tendency toward agreeableness (mean: 15.77 out of 16, SD: 2.91)

and conscientiousness (mean: 15.68 out of 16, SD: 2.59), and lower in neuroticism (mean: 10.67 out of 16, SD: 3.54) (table 1).

3.2 HEALTH BEHAVIORS OF SAMPLE AT BASELINE AND DURING STUDY

The majority of participants reported moderate levels of daily physical activity at baseline (69.80%). Over the course of the observed period, most participants reported exercising (84.90%) and drinking caffeine (84.90%) on at least one day, while cigarette smoking was uncommon (20.80%). The sample was largely split on alcohol intake (47.20% reported alcohol use on at least one day of the observed period) (table 2).

3.3 VASOMOTOR SYMPTOMS

3.3.1 Baseline

During the telephone screen for eligibility, participants reported having typically frequent daily vasomotor symptoms (24.50% reporting 1-3 in a typical 24 hour period compared to 41.50% reporting 6 or more during that period), which they generally reported to be mild to moderate in severity (67.90%) and moderately or very bothersome (84.90%). During the initial study visit, participants reported only moderate daily interference by their vasomotor symptoms (mean: 4.03 out of 10, SD: 2.71) (table 3).

3.3.2 Daily diaries

All participants had at least one self-reported vasomotor symptom during the observed period, with participants diary-reporting 7.39 (SD: 5.88) vasomotor symptoms per day on average. Diary reporting suggests that retrospective reporting of typical frequency may have been underestimated, with fewer participants reporting a mean of 3 or fewer vasomotor symptoms during a day of observation (18.90% in diaries vs. 24.50% in telephone screen), and more reporting a mean of 6 or more each day (66.00% in diaries vs. 41.50% in telephone screen). Participants reported a rate of fewer than one hot flash per hour awake (mean: 0.44, SD: 0.41), and a slightly lower rate of night sweats per hour asleep (mean: 0.34, SD: 0.28). When broken down into sleep and wake vasomotor symptoms, participants reported more daily hot flashes on average (mean: 5.42, SD: 4.70) than night sweats (mean: 1.98, SD: 1.61). Participants generally reported mild to moderate vasomotor symptom severity (mean: 1.37, SD: 0.48) and moderate bother (mean: 2.21, SD: 0.60) (table 3).

3.3.3 Actigraphy

Participants also used the event marker button on their study Actiwatches to report vasomotor symptoms. The frequency reported using this method was commensurate with that of the diaries; 6 or more occurrences within each 24 hour period were reported by most women over the observed period (73.10%). Given the similarities in this data and in order to best match vasomotor frequency reporting to vasomotor symptom severity and bother reporting, event marker data was not used in the current analyses (table 3).

3.4 NEGATIVE AFFECT

3.4.1 Baseline

Participants generally reported depressive (CESD mean: 13.53, SD: 8.68) and anxiety (ie, STAI-trait mean: 36.45, SD: 9.34) symptom scores well below conventional cut-offs for clinical significance (table 4).

3.4.2 Daily diaries

Participants generally reported minimal levels of negative affect (PANAS-negative affect subscale mean: 12.10, SD: 3.71, range: 10-50; PANAS-sadness subscale mean 6.55, SD: 3.06, range: 5-25; Gibson scale mean 4.30, SD: 1.55, range: 3-15), and moderate levels of positive affect (PANAS-positive affect subscale mean: 24.70, SD: 7.18, range 10-50) (table 4).

3.5 SLEEP ASSESSMENT

3.5.1 Baseline

Assessment of sleep parameters by the Pittsburgh Sleep Quality Index (PSQI) suggested largely problematic sleep in the sample, typical of the general population of women in this age range. A majority of participants (n=38, 71.70%) had PSQI total scores over 5, indicative of poor sleep; the mean total score for the sample was over the clinical cut-off for poor sleep (mean: 8.06, SD: 3.16). Over half of the sample (n=30, 56.60%) reported typically sleeping for fewer than the

recommended 7 hours a night, while under half of the sample reported achieving the suggested sleep efficiency of 85% or more of time in bed spent actually asleep (n=23, 44.20%) (table 5).

3.5.2 Daily diaries

Participants generally reported that they felt moderately rested (mean: 3.93, SD: .93, range: 1-6) and had a moderately good night's sleep (mean: 3.75, SD: .92, range: 1-6) the previous night during their morning diary entries. However, most women (90.60%) reported that they had trouble sleeping the previous night during their morning diary entry on at least one day during the observed period (table 5).

Naps were also commonly reported, with an average of 2.56 (SD: 2.89) naps occurring over the observed period. The majority of women (64.20%) reported taking at least one nap during at least one day of observation, and naps were reported on an average of 30% of days over the observation period. Over 30% of women reported taking more than one nap on at least one day of observation (table 5).

3.5.3 Actigraphy

Sleep parameters measured by actigraphy generally suggested that participants overestimated their typical sleep duration and quality in the PSQI self-report during the baseline visit. Actigraphy-measured sleep duration was less than the recommended 7-8 hours/night (mean: 5.63, SD: 0.98). Participants exhibited over an hour of WASO on average (mean: 63.57 minutes, SD: 36.10). It should be noted that participants, on average, reported sleep efficiency

far below the optimal 85% sleep efficiency threshold (overall mean: 75.95%, SD: 11.24) (table 5).

3.6 CORTISOL

3.6.1 Salivary cortisol

Salivary cortisol values suggested that, as expected, participants had higher morning (mean 16.82 nmol/l, SD: 7.50) than bedtime (mean 3.96 nmol/l, SD: 4.00) cortisol levels averaged across all three days of sampling, consistent with a negative diurnal slope. After adjusting for the self-reported time between morning and bedtime collection, mean diurnal slopes of salivary cortisol were negative, though not particularly steep (mean slope: -0.97, SD: .79). Overall mean cortisol exposure for each day was commensurate with morning and bedtime values (mean daily exposure: 10.46 nmol/l, SD: 4.46) (table 6).

3.6.2 Hair cortisol

Hair cortisol values fell within expected values (mean: 3.50 nmol/l, SD: 5.58). Values were log-transformed for analyses due to non-normality of the distribution (table 6).

3.7 EXPLORATORY ANALYSES: DIFFERENCES BY RACE/ETHNICITY

Though not by design, the demographic make-up of the final sample allowed for direct comparison of primary variables by race/ethnicity. Some significant differences between Caucasian and African-American participants were noted, and are detailed in the following paragraphs.

In between-women comparisons at baseline, African-American participants had greater BMI and waist circumference measures, while Caucasian participants reported higher levels of educational attainment and better self-rated health (table 1). In contrast, no racial/ethnic differences were seen in the measures of personality assessed during the participants' initial study visit.

During the initial phone screen, African-American and Caucasian participants differed only in their ratings of vasomotor symptom severity, with African-American women being more likely to rate them as typically severe (53.80%) compared to the Caucasian participants (11.10%) (table 3). In daily diaries, while there were no significant racial/ethnic differences in hot flash frequency, severity, or bother, African-American participants were more likely to report a higher nightly frequency of night sweats.

In questionnaires, no racial/ethnic differences were seen in the measures of affect assessed during the participants' initial study visit. Diary-reported negative affect did not differ, but African-American women, on average, reported higher positive affect scores during their diary ratings (table 4).

Sleep issues seemed to be particularly prevalent for the African American participants in this sample. On the PSQI, African-American participants were significantly less likely to report 7 or more hours a sleep at night, less overall sleep, and higher PSQI total scores. Though sleep

duration did not significantly differ between the Caucasian and African-American participants, all other actigraphy-measured sleep parameters suggested significant race/ethnic differences. African-American women had more minutes of wake after sleep onset, more frequent nocturnal wakening, and less sleep efficiency compared to Caucasian participants (table 5).

Although there were no significant overall differences in mean daily average salivary cortisol or diurnal salivary cortisol slope by race/ethnicity, African-American participants exhibited significantly higher bedtime cortisol values than Caucasian participants. Race/ethnicity differences were also seen in hair cortisol values, with African American women exhibiting higher levels of cortisol concentration than their Caucasian counterparts (table 6).

3.8 SPECIFIC AIMS

3.8.1 Specific Aim 1: Examine the contribution of VMS to within-day and next-day negative affect.

It was hypothesized that diary-reported vasomotor symptoms would be positively associated with diary-reported negative affect on the same day. This hypothesis was supported. Specifically, in models of observations nested within days, nested within women, daily PANAS negative affect as reported in daily diaries was positively associated with vasomotor symptom bother ($B=1.46$, $p<0.001$), and daytime hot flash bother ($B=1.46$, $p<0.001$) as reported in daily diaries on the same day, even after adjusting by demographic and health behavior-related variable. Within days, daily PANAS sadness as reported in daily diaries was positively associated with vasomotor symptom bother (overall during each 24 hour period; hot flashes and

night sweats combined) (B=0.46, p=0.04) and daytime hot flash bother (B=0.46, p=0.04) as reported in daily diaries on the same day, even after adjusting by demographic and health behavior-related variables. Within days, daily Gibson negative affect as reported in daily diaries was positively associated with vasomotor symptom bother (B=0.44, p<0.01), and daytime hot flash bother (B=0.44, p<0.01) as reported in daily diaries on the same day, even after adjusting by demographic and health behavior-related variables (table 7).

It was hypothesized that diary-reported vasomotor symptoms would be positively associated with diary-reported negative affect the following day. This hypothesis was supported. Specifically, in models of observations nested within days, nested within women, next day PANAS negative affect in daily diaries was predicted by the previous day's diary reported hot flash frequency rate (B=0.18, p=0.05, trend), and diary reports of the previous night's night sweat severity (0.80, p=0.02) and bother (0.61, p=0.02), even after adjusting for demographic variables, health behaviors, the previous day's ratings of PANAS negative affect, and ratings of hot flash frequency rate (for model examining previous day hot flash frequency rate), hot flash severity (for model examining previous night's night sweat severity), or hot flash bother (for model examining previous night's night sweat bother) on the day commensurate with affect ratings as outcome. The relationship seems to specifically follow the pattern of vasomotor symptoms one day contributing to negative affect the following day; when next day hot flash frequency rate is entered as the outcome in an otherwise equivalent model, no relationship is seen between previous day negative affect and next day hot flash frequency rate (B=0.01, p=0.33). Next day Gibson negative affect in daily diaries was also associated with the previous night's night sweat severity (0.62, p<0.01) and bother (0.35, p=0.03), even after adjusting for demographic variables, health behaviors, the previous day's ratings of Gibson negative affect,

and ratings of hot flash severity (for model examining previous night's night sweat severity), or hot flash bother (for model examining previous night's night sweat bother) on the day commensurate with affect ratings as outcome (table 8).

3.8.2 Specific Aim 2: Examine sleep as a mediator of the association between VMS and next-day negative affect.

It was hypothesized that sleep disruption, as measured by actigraphy, would partially explain the relationship between vasomotor symptoms and following day negative affect, as measured by diary reporting. In models of observations nested within days, nested within women, the role of sleep as a mediator of the association between vasomotor symptom experience and next-day negative affect was not supported in this sample. The frequency rate of night sweats reported was not associated with next day negative affect, and therefore not further assessed. Night sweat bother as reported the morning after actigraphy was associated with next day Gibson negative affect (B=0.29, p=.02), but equivalent with the introduction of sleep duration or sleep efficiency in the model. There was a slight increase in this coefficient with the introduction of WASO into the model (B=0.32, p=02). Similar results were seen in the relationship between night sweat severity and next day negative affect. Night sweat severity as reported the morning after actigraphy was associated with next day PANAS negative affect (B=0.78, p=0.01) and next day Gibson negative affect (B=.48, p<.01). These coefficients increased slightly with the addition of sleep duration (PANAS, B=.86, p=0.01; Gibson, B=.51, p<.01), sleep efficiency (PANAS, B=.86, p=0.01), and WASO (PANAS, B=.85, p=0.01; Gibson, B=.53, p<.01), suggesting that the relationship between night sweat severity and negative affect is independent of sleep parameters, and even strengthened when sleep parameters are held constant (Table 9).

3.8.3 Specific Aim 3a: Examine diurnal cortisol slope and hair cortisol as physiologic measures associated with negative affect, vasomotor symptoms, and sleep parameters.

It was hypothesized that diurnal cortisol slope would be positively associated with negative affect. This hypothesis was supported. As expected, in models of daily observations nested within women, diurnal cortisol slope was significantly and positively associated with measures of negative affect (PANAS NA B=0.68, p=.01; PANAS Sadness B=0.43, p<.001), such that a flatter diurnal cortisol slope was related to higher negative affect scores. Mean daily cortisol exposure also showed a significant association with PANAS sadness (B=0.07, p=0.03). These relationships held in fully adjusted models, including demographic variables and assessment of daily diary-reported health behaviors (table 10). In models of hair cortisol values and overall mean negative affect values for each woman, no significant relationships with transformed or non-transformed hair cortisol and negative affect variables were exhibited in unadjusted or fully adjusted models (data not shown).

It was hypothesized that diurnal cortisol slope would be positively associated with vasomotor symptoms. This hypothesis was supported. In models of daily observations nested within women, diurnal cortisol slope was significantly and positively associated with some measures of vasomotor experience, such that a flatter slope was related to higher vasomotor symptom ratings. Specifically, a flatter diurnal cortisol slope was related to increased reports of vasomotor symptom severity (B=0.06, p=0.02), hot flash severity (B=0.09, p=0.03), and hot flash bother (0.10, p<0.01). These relationships held in fully adjusted models, including demographic variables and assessment of daily diary-reported health behaviors (table 11). In models of hair cortisol values and overall mean vasomotor symptom values for each woman, higher hair cortisol values were associated with overall increased daily hot flash frequency rate

(B=0.05, p=0.01), even after adjusting for demographic and health behavior-related variables (table 11).

It was hypothesized that diurnal cortisol slope would be associated with sleep parameters, such that a flatter slope would be related to measures of poor sleep. This hypothesis was partially supported. Specifically, in models of mean daily observations nested within women, shorter sleep duration (B=-0.38, p<0.01) but less time awake after sleep onset (B=-7.62, p=0.02) was seen the night prior to a flatter diurnal cortisol slope. Longer sleep duration (B=.40, p=.04) was observed the night following a flatter diurnal cortisol slope. This relationship held in fully adjusted models, including demographic variables and assessment of daily diary-reported health behaviors. Higher mean cortisol exposure over the observed period was associated with more time awake after sleep onset (B=2.68, p=0.02) and reduced sleep efficiency (B for log-transformed variable=-.004, p=0.03). These relationships held in fully adjusted models, including demographic variables and assessment of daily diary-reported health behaviors (table 12). In models of hair cortisol values and overall mean actigraphy-measured sleep values for each woman, no significant relationships were seen in unadjusted or fully adjusted models (data not shown).

3.8.4 Specific Aim 3b: Examine cortisol as a mediator linking vasomotor symptoms with negative affect.

It was hypothesized that a flatter diurnal cortisol slope would partially explain the relationship between vasomotor symptoms and same day negative affect. This hypothesis was supported. Mediation was then tested with two-level hierarchical models using the Krull-MacKinnon method (Krull & MacKinnon, 1999). In the first step (1), negative affect was predicted from

same day VMS with two-level hierarchical linear regression. In the second step (2), cortisol slope was included in the regression model in order to estimate negative affect from both VMS and cortisol (A). The difference between the estimates of the VMS variable in the first and second equation estimated the extent to which cortisol accounts for the relationship between VMS and same day negative affect. Specifically, in models of mean daily observations nested within women, the significant association between hot flash frequency rate and measures of negative affect was not affected by the inclusion of diurnal cortisol slope in the model (Gibson NA: -.31, p=0.04). However, hot flash severity and measures of negative affect remained significant but were reduced when diurnal cortisol slope was introduced into otherwise equivalent, fully adjusted models (PANAS NA: HF severity B=2.28, p<0.01, with diurnal slope, B=2.16, p<0.01; Gibson NA: HF severity B=0.83, p<.001, with diurnal slope, B=0.61, p=.048). When examining diurnal cortisol slope as a mediator linking hot flash bother and measures of negative affect, there was no significant change in the coefficients for PANAS NA (B=1.73, p<.01), but a relatively large change in another measure, with hot flash bother no longer significantly associated with negative affect after including diurnal cortisol slope in the model (Gibson NA: B=0.49, p=0.04, with diurnal slope, B=0.28, p=0.17) (table 13).

Mediation was also tested with aggregate variables in linear regression using the Sobel method ((Sobel, 1982). No significant results were found using this model, with cortisol and negative affect compared between women (data not shown). As hair cortisol was not associated with negative affect or vasomotor symptom experience in independent models, further mediational analyses were not conducted using these parameters.

3.9 EXPLORATORY ANALYSES: DIFFERENCES BY RACE/ETHNICITY

The ability to fully explore moderation by race/ethnicity in the specific aims presented above was limited by the relatively small sample and large number of relevant covariates. However, exploratory analyses suggested some differences in the pattern of results by race/ethnicity that merit additional study.

The relationship between vasomotor symptom experience and same day negative affect (specific aim 1a) may be strongest among African American participants. Specifically, effect modification was seen in the relationship between vasomotor symptom bother and PANAS negative affect by race (interaction term $p<.01$), and for hot flash bother and Gibson negative affect by race (interaction term $p=0.04$). This relationship was also seen in the relationship between vasomotor symptoms and next day negative affect (specific aim 1b), such that hot flash bother more strongly predicted next day Gibson negative affect among African American participants than Caucasian participants (interaction term $p=0.04$). Exploratory moderation analyses by race/ethnicity suggest that the relationship between a flatter cortisol slope and daytime hot flash severity (Specific Aim 3a) is stronger among the African American participants (interaction term $p=.04$). No other differences in relationships by race/ethnicity were seen with other vasomotor symptom-related outcomes.

4.0 DISCUSSION

This study aimed to examine the within day and day-to-day relationships between vasomotor symptoms and negative affect, and the potential role of sleep disturbance and cortisol dysregulation in these relationships, using ambulatory and physiological data among a sample of relatively healthy women in midlife. After accounting for a number of health and demographic variables, it was found that women reported more negative affect on both the same day and the day following a more negative experience of vasomotor symptoms. A flatter diurnal cortisol slope partially explained the same day relationship between vasomotor symptoms and negative affect, while sleep disturbance did not appear to play a role in linking previous day hot flashes or night sweats to next day negative affect.

Participants reported more negative affect on the days that they also reported more bothersome vasomotor symptoms. While an association between vasomotor symptoms and negative affect has been commonly found in a growing literature (Blümel et al., 2004; Bromberger et al., 2003, p. 20; Bromberger & Kravitz, 2011;Bromberger et al., 2001, p. 200; Burleson et al., 2010; Collins & Landgren, 1994; Freeman et al., 2005; Gibson et al., 2011; Gold et al., 2006; Hunter, Gupta, Papitsch-Clark, & Sturdee, 2009; Ishizuka et al., 2008; Joffe et al., 2002; Li, Yu, Ma, Sun, & Yang, 2008; Thurston et al., 2008, 2005), most studies have examined only vasomotor symptom occurrence, with both vasomotor symptom and mood reporting limited by retrospective recall. The findings of the current study extends the prior literature by utilizing

multiple aspects of vasomotor symptom experience and negative affect, and measuring each with ambulatory data to capture self-reported experiences shortly after they occur throughout the daily lives of midlife participants. Given the common prevalence of vasomotor symptoms among women in midlife, understanding the aspects of vasomotor symptom experience that may contribute to mood disturbance among symptomatic women is essential for informing intervention and research efforts. Further, understanding the mechanisms underlying the association between negative affect and vasomotor symptom experience in the daily lives of women in midlife may help to inform intervention efforts to improve quality of life and health among symptomatic women throughout the menopausal transition.

In a novel finding, this study suggests that cortisol dysregulation is related to vasomotor symptoms, and may play a role in the daily relationship between vasomotor symptom experience and negative affect. Specifically, vasomotor symptom severity and bother, though not frequency, were associated with flatter diurnal cortisol slopes in the current study. This finding contributes to the previously sparse empirical evidence linking vasomotor symptom severity (Cagnacci et al., 2011; Woods et al., 2006) and occurrence (Cignarelli et al., 1989; Genazzani et al., 1984; Meldrum et al., 1984) with cortisol dysregulation, and improves upon past methodological limitations including use of urinary cortisol (Cagnacci et al., 2011; Woods et al., 2006), a single day of salivary cortisol measurement (Sievert, 2012), and very small sample sizes (Cignarelli et al., 1989; Genazzani et al., 1984; Meldrum et al., 1984). Consistent with past studies (Heaney et al., 2010; Knight, Avery, Janssen, & Powell, 2010), diurnal cortisol slope in this sample was also flatter on average among women who reported more negative affect. The interrelationships between these factors were further investigated, and it was found that a flatter diurnal cortisol slope appears to explain some of the variance in the relationship between self-

reported vasomotor symptom severity, bother, and negative affect in the daily life of symptomatic women.

These findings may lend support to the hypothesis that as an unpredictable stressor often occurring in social contexts, and anecdotally reported as embarrassing and highly noticeable (Smith et al., 2011), vasomotor symptoms may trigger stress-related cortisol release and higher overall cortisol exposure across the day (Meldrum et al., 1984). Specifically, vasomotor symptoms that are perceived as severe or bothersome are more likely to be processed as a socio-evaluative threat, the type of stressor known to most strongly elicit cortisol reactivity (Dickerson et al., 2008). High levels of cortisol reactivity and consequent high levels of cortisol exposure are thought to result in the dysregulation of diurnal cortisol rhythms (Woods et al., 2006) linked to negative affect (Knight et al., 2010). Vasomotor symptoms that are perceived as bothersome may therefore elicit stress-related cortisol release, contributing to negative affect and cortisol dysregulation over time.

Though these findings contribute support to a theoretical model of negative perception of vasomotor symptom experience contributing to cortisol reactivity, many assumptions remain. In the current study, reactivity was not studied, and can only be assumed to play a role in the proposed fashion. As analysis was limited to the nested and aggregate mean of cortisol slope and negative affect on the days of salivary sample collection, the direction of effect cannot be determined. Further, investigation of overall cortisol exposure as measured by mean daily salivary cortisol exposure and hair cortisol, presumed to be an important factor in cortisol dysregulation, did not show the same patterns of associations with vasomotor symptoms or negative affect as diurnal slope. The theoretical pathway linking daily cortisol dysregulation to daily negative affect is tenuous at best, and remains to be clarified. Alternative pathways, such

as circadian dysregulation underlying cortisol dysregulation as well as vasomotor symptoms and negative affectivity, may also be considered. The findings of the current study therefore provide a novel contribution to our knowledge of the relationship between cortisol and vasomotor symptoms, and are promising for further understanding the complex relationship between vasomotor symptom experience and negative affect in daily life, while also raising a number of questions to be pursued in future research.

In addition to co-occurring on the same day, vasomotor symptom experience was associated with increased next day negative affect. Specifically, women reported more negative affect the day after reporting more frequent hot flashes, and after reporting more bothersome or severe night sweats the previous night. These relationships were seen even after adjusting for previous day negative affect and same day vasomotor symptoms, in addition to demographic and health behavior-related variables. Temporal relationships between vasomotor symptoms and mood, particularly on a daily basis, have rarely been investigated. These findings contribute to this limited literature by replicating and extending those of Burleson et al. (Burleson et al., 2010) and Gibson et al. (Gibson et al., 2011), which found that the occurrence of any vasomotor symptoms was associated with next day negative affect, independent of negative affect the previous day and vasomotor symptom reporting the same day. While the previous studies had limited measures of both affect and the presence or absence of undifferentiated hot flashes or night sweats in the previous 24 hours, these findings allow for investigation of the role of hot flashes compared to night sweats, as well as different aspects of vasomotor symptom experience, in relationship to commonly used measures of negative affect.

It had been hypothesized that the relationship between vasomotor symptoms and next day negative affect was driven by the experience of night sweats during the intervening night, a

model known as the domino hypothesis (Joffe et al., 2009). The contribution of self-reported vasomotor symptoms to perceived sleep disturbance among women in midlife has been supported in a number of cross-sectional studies, and disturbed sleep is known to impact psychological functioning and next-day mood. However, an association between self-reported or physiologically measured night sweats and objective measures of sleep, essential to this hypothesis, has not been well-supported by empirical evidence. Similarly, the frequency of self-reported night sweats was not related to actigraphy-measured sleep parameters in the current study, as would be expected if the occurrence of night sweats was disruptive to sleep. It therefore does not appear that the night sweats themselves contributed to sleep disturbance in this sample. In contrast to reported night sweat frequency, subjective ratings of night sweat severity and bother, as reported the following morning, were related to actigraphy measures of sleep duration and sleep efficiency. These sleep parameters were also associated with next day negative affect; mediational analyses suggest that they do not explain the relationship between night sweat bother or severity and next day negative affect.

A number of factors may explain this pattern of findings. Foremost, information on night sweats derived from self-report may not be highly representative of women's experience of these symptoms. As reporting occurs in the morning following awakening, self-report necessitates a longer period of retrospective recall than the frequent within-day reporting schedule. Additionally, accuracy of self-report is coupled with the complication of potential sleepiness at the time of reporting, as well as at the time of symptom occurrence, which may affect recall. Women may not have been aware of or remember events with onset during sleep, with the exception of those events that were particularly disruptive (commensurate with higher severity rating as defined) or bothersome (assuming that bother is related to wakefulness). Alternatively,

over-reporting or more negatively biased reporting of the subjective experience of night sweats during the previous night may result from having had a poor night's sleep. Regardless of occurrence, women may report a more negative experience of overnight vasomotor symptoms as attribution to their wakefulness the previous night, current lack of feeling rested, and impaired psychological functioning.

Actigraphy-measured sleep parameters were also related to cortisol slope, both the night prior to and the night following measurement. Specifically, a flatter diurnal cortisol slope was related to shorter sleep duration, though less time awake after sleep onset, the night prior to cortisol sampling. That is, after a night of less sleep, women exhibited flatter diurnal cortisol slopes. This is consistent with past studies, in which a flatter diurnal slope is related to self-reported shorter sleep as well as greater sleep disruption in a sample of midlife men and women (Kumari et al., 2009), and may be related to findings that sleep deprivation contributes to elevated resting cortisol levels and exaggerated cortisol response to a laboratory-based socio-evaluative stress task (Minkel et al., 2014). It is possible that shorter sleep as measured by actigraphy may set the stage for both cortisol dysregulation and negative affect, exacerbating the role of vasomotor symptom bother and severity in contributing to both during the day. However, after a day in which women exhibited flatter diurnal cortisol slopes, they had longer sleep. Overall cortisol exposure was associated with less efficient sleep and more time awake after sleep onset. The complex and likely bidirectional relationships between these behavioral and physiological factors in midlife remain unclear, and warrant future investigation.

The experience of vasomotor symptoms and negative affect, and the factors that link them, may differ among women from different racial/ethnic backgrounds. The study sample was fairly equally split between African American and Caucasian women, allowing for the

opportunity to conduct exploratory analyses on race/ethnic differences on the significant findings within each specific aim. Most importantly, stronger relationships between vasomotor symptom bother and both same day and next day negative affect reporting were seen in African American participants compared to their Caucasian peers. Though past studies have suggested that African American women report vasomotor symptoms as more bothersome (Thurston et al., 2008), this is the first known study to demonstrate moderation by race/ethnicity of the relationship between daily measurements of negative affect and vasomotor symptom experience. These relationships were not seen to vary by race/ethnicity in Gibson et al. (Gibson et al., 2011), perhaps because only the presence/absence of vasomotor symptoms, and not the subjective experience of vasomotor symptom bother, were measured in that study.

Additionally, a novel finding concerns the association found between diurnal cortisol and vasomotor symptoms. As mentioned previously, there is very little empirical evidence surrounding the proposed relationship between cortisol and vasomotor symptoms, and the few studies that have investigated this relationship did not examine, or have sufficient sample composition to examine, moderation by race/ethnicity. However, findings from the current study suggest that the relationship between hot flash severity and a flatter diurnal cortisol slope may be stronger among African American than Caucasian participants. The relationship between hot flash severity and cortisol seen in the sample may be due to the patterns of severity reporting in these two groups, which has a more restricted range and overall more likely to be reported as mild among the Caucasian women compared to the African American women in this sample. It may also reflect a mechanism by which African American women in the menopausal transition have an increased risk of the cardiovascular risk factors thought to be related to cortisol dysregulation and hypercortisolemia; that is, African American women are more likely to report

more severe hot flashes, which among that group are more likely to contribute to unhealthy cortisol patterning. There is a limited ability to determine temporality in this data; it is also possible that the flatter cortisol slopes seen among the African American women in this sample may play a role in increasing hot flash severity due to underlying effects on the hypothalamic-pituitary-ovarian axis (Woods et al., 2006). These findings should be interpreted with an abundance of caution given concerns about sample size and power to determine interactions by race/ethnicity, but as much remains unknown about race/ethnic differences between risk factors and health outcomes as pertains to the menopausal transition, they yield interesting hypotheses to be pursued in future research.

A number of other findings are worth mentioning relating to risk for negative affect, vasomotor symptoms, cortisol dysregulation, and poor sleep in this sample. In the overall sample, women with higher levels of educational attainment were less likely to report negative affect the same and following day, while cigarette smoking during the observed period was related to increased negative affect reporting in some models. Cigarette smoking had an even stronger impact on next day negative affect reporting. Self-rated health was also protective against some measures of same day and next day negative affect reporting in some models. Surprisingly, self-reported exercise during the observation contributed to next day negative affect. Sleep duration was reduced among with higher levels of education and among postmenopausal women compared to perimenopausal women; postmenopausal women spent less time awake after sleep onset. Women who reported less than excellent health slept longer than those in excellent health, though exhibited more fragmented sleep and more time spent awake after sleep onset. Both sleep duration and WASO were measured as reduced on nights following reported alcohol use, limited to only a yes/no response; after drinking, women slept less, but also

spent less time awake after they fell asleep. Though the majority of these demographic and health behaviors exhibited expected relationships with vasomotor symptoms, negative affect, and sleep parameters, the replication of these associations with ambulatory data collection provides useful information about the role of these factors in the daily life of women in midlife.

This study had some limitations that warrant mention. Eligibility criteria mandated that women have daily vasomotor symptoms, and not currently be taking any psychotropic medications, cortisol supplements or medications, or hormone therapy. Additionally, participants who completed the study had to be both willing and able to come to the study site and be compliant with frequent entries in an electronic daily diary over the course of a week, as well as wear an Actiwatch and collect saliva samples on a time-dependent schedule. Participation therefore required that women be relatively healthy and possess a high level of motivation and compliance, which may not be representative of all women in midlife. It should also be noted that this sample was unique; participants reported poor sleep and frequent naps, perhaps reflective of the high level of unemployment and inconsistent daily schedules indicated by many in the sample. Commensurate with the demands on participant's time for the week of the study that participation involved, issues related to missing data were a concern. Missing data was primarily problematic with saliva and hair cortisol samples, due to equipment failure, user error or ineligibility, and participant forgetfulness. This was particularly true of hair cortisol, which was collected from only a subset of participants who were both willing and had hair long enough to sufficiently participate in this optional aspect of the protocol. Therefore, the full scope of experience may not have been adequately captured in this study design, and data may be biased toward days in which participants had fewer stressors and negative events interfering with their compliance. The overall model, specified a priori, includes assumptions about the temporality of

relationships between vasomotor symptom experience, cortisol, and negative affect, which may not be an accurate reflection of the relationship between these factors. Additionally, unmeasured variables (ie, employment status/history, social support, marital status) may play important roles that cannot be determined in the absence of their inclusion. Finally, the sample size of the study, while powered to capture any primary associations between diary-reported vasomotor symptoms and negative affect if they occurred, may limit the ability to fully interpret all aims. This is particularly true of exploratory analyses by race/ethnicity, and analysis on limited samples such as those with complete salivary and/or hair cortisol data available.

Despite these limitations, this study also had considerable strengths. Foremost among these is the use of electronic daily diaries for ambulatory assessment of vasomotor symptoms and negative affect. Though the technical competence and compliance required of these measures may limit generalizability, utilization of these technologies presents a major advancement from the typical retrospective recall, likely colored by mood in the moment and subject to failures of memory, of most studies assessing vasomotor symptoms. Further, this allowed for nuanced collection of multiple aspects of symptom and mood experience, including temporal variation, to be explored in future study, aspects of vasomotor symptom experience including severity and bother in addition to frequency, and the ability to separate night sweats from daytime hot flashes. A wide array of mood items, asked on a Likert scale, allowed for the construction of multiple mood measures and was designed to reduce response bias. Similarly, the use of actigraphy in addition to self-reported sleep evaluation allowed for a more objective measure of a variety of sleep parameters. The length of the study, 7 days of observation, was designed around the greatest validity for these actigraphy-based sleep measures.

An additional strength of this study was the use of three days of cortisol sampling to determine a mean diurnal slope and mean affective measures for each participant across several days, given the known day-to-day variability in these measures (Segerstrom, Boggero, Smith, & Sephton, 2014; Whitehead & Bergeman, 2014). The utilization of novel hair cortisol assaying techniques further adds to our knowledge of the relationship between cortisol and a number of factors, and may better get at chronic cortisol exposure rather than salivary or urinary cortisol's short-term snapshot. Further, hair collection avoids the possibility of participant forgetfulness and error inherent in time-dependent saliva sample collection. Finally, although the sample size was too small too thoroughly and confidently assess interactions by race/ethnicity, the racial composition of the sample allowed for some investigation of the differing relationships by race between these common aspects of the menopausal experience, further informing our knowledge in this important area.

The current study contributes to our understanding of some aspects of the relationship between mood and menopausal symptoms, while raising additional questions for future study. A number of additional avenues remain to be addressed using the data collected for this study, and not included in analyses of these primary aims. Foremost among these are an investigation of the roles of various individual differences, including personality factors; the role of both self-reported typical health behaviors in conjunction with actigraphy and diary reporting; questionnaire-based assessments of typical negative affect in conjunction with diary reporting of daily negative affect; and reported duration of vasomotor symptoms; in the relationships seen between vasomotor symptoms and negative affect. Additional factors that were assessed and remain to be explored include the role of napping, measures of positive affect, and attitudes toward aging and menopause. The hypothesis that vasomotor symptoms are processed as

socioevaluative threat, contributing to cortisol response, can be further investigated with examination of questionnaires assessing the perceived impact of vasomotor symptoms on daily life, as well as diary data about social interactions and patterns throughout the day. Alternately, the role of underlying circadian rhythm dysregulation as a contributor to both cortisol dysregulation and VMS may be explored in part with the data collected in this study. Uniquely, the ambulatory data collected can allow for additional examination of the patterns of temporal variation in vasomotor symptoms, mood, and the relationships between them, with an eye to investigating the circadian patterns of these factors and how they relate to other circadian rhythms, such as sleep and social rhythms as reported in the diaries. A number of relevant factors collected in the current dataset remain to be explored in a continuing effort to better understand the relationships between vasomotor symptom experience, negative affect, and health among women in midlife.

The findings of this study suggest that vasomotor symptom bother is the key factor related to various aspects of affect and health. Clinical research is needed to determine whether interventions can shift women's subjective perceptions of their vasomotor symptom experience, and if reductions in perceptions of bother rather than frequency in turn improve the affective and physiological stress response to their occurrence. Given the importance of these factors for quality of life as well as in the development of cardiovascular risk factors, intervention efforts should focus not on reducing symptom frequency, but on reframing women's attitudes towards symptoms themselves.

Such intervention studies may also include psychoeducation around sleep to improve sleep hygiene and patterns for affected women, reframing previously held beliefs that night sweats cause poor sleep which may get in the way of women taking ownership over their

problematic sleep patterns and how to address them. Offering psychoeducation on a more nuanced view of the relationship between mood and menopausal symptoms and women's perceptions of sleep quality may offer an opportunity to improve all. Additionally, advances in mobile technology provide improved ability to assess or possibly influence women's perception of hot flashes/night sweats as they occur, and examine concomitant changes in mood and stress response, in order to improve both data collection and intervention dissemination. Further, the use of mobile technology may provide a venue to help women empower themselves to take control of the impact that these common symptoms have on their lives and their health, while also providing the opportunity to advance research on the role of health behaviors and lifestyle modification on symptom reduction.

In summary, using ambulatory methods to fully characterize and relate daily vasomotor symptom and affective experiences of women across a typical week, actigraphy to assess sleep parameters independent of reporting bias, and both hair collection and three days of saliva sampling to examine cortisol dysregulation and exposure, this study aimed to improve our understanding of the temporal relationships between these factors and the role of health behaviors and biological mechanisms that may link them. In the current study, women reported more negative affect on the same day that they also reported more frequent or bothersome vasomotor symptoms, and on the day following a night in which they experience severe and/or bothersome night sweats. The relationship between same day vasomotor symptom experience and negative affect may be partially explained by cortisol dysregulation, with cortisol stress response possibly triggered by the subjective experience of vasomotor symptoms perceived to be bothersome. The relationship between vasomotor symptoms, and specifically night sweats, and next day negative affect remains to be clarified. Sleep disturbance is independently related to

bothersome or severe night sweats and next day negative affect, but does not explain the relationship between night sweats and next day negative affect. The interrelationship of these factors may rest on subjective sleep quality, colored not by the disruptive occurrence of night sweats, but by the negative attributions of the experience of those symptoms experienced by many women. These findings may have implications for both research and clinical applications, with an emphasis on shifting attention away from concern about vasomotor symptom occurrence and frequency, and toward understanding and reducing symptom bother among women in midlife to improve quality of life and potential downstream effects on mood, health, and physiologic function.

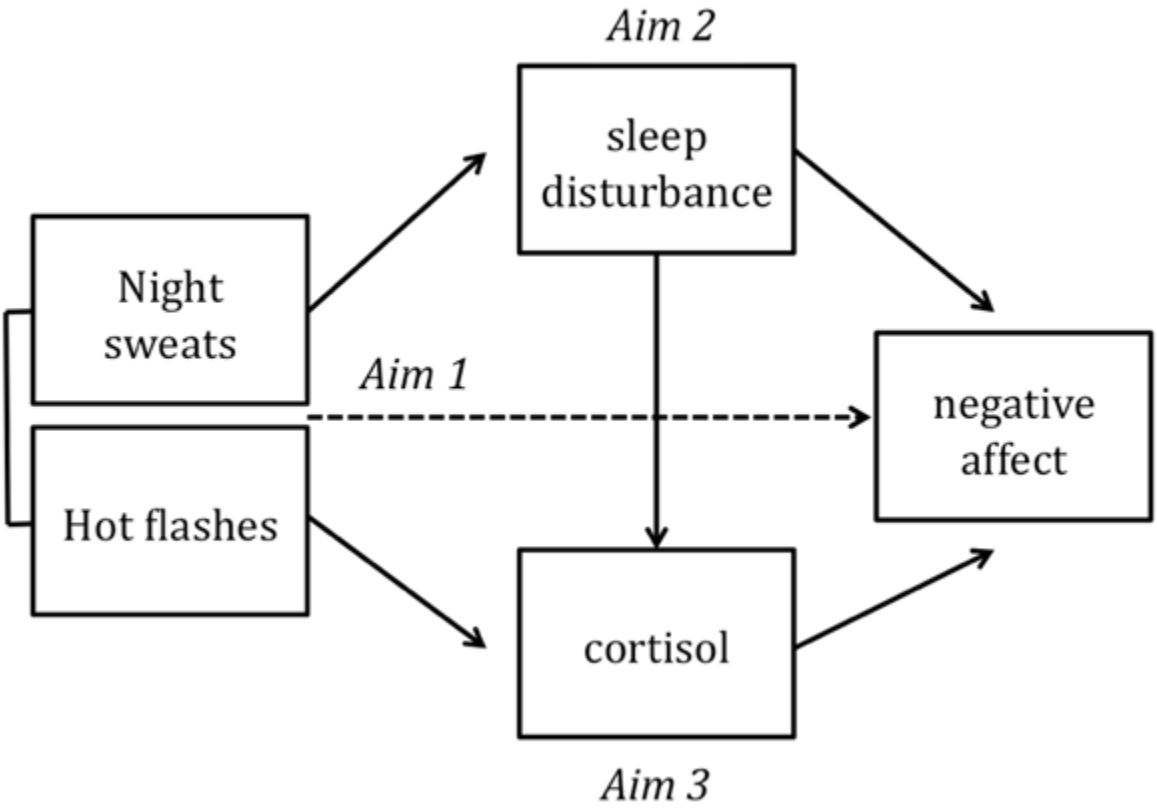

Figure 1. A priori model with disturbed sleep and cortisol dysregulation linking VMS and negative affect.

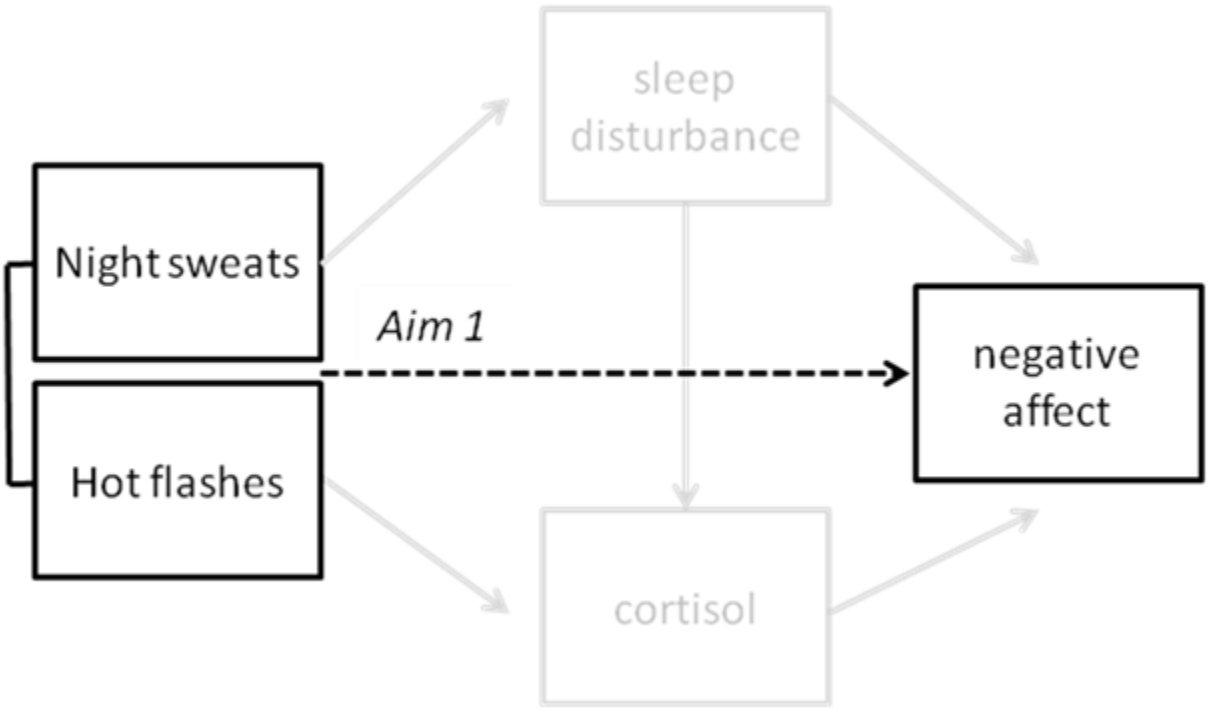

Figure 2. Aim 1: VMS predicting same-day and next-day negative affect

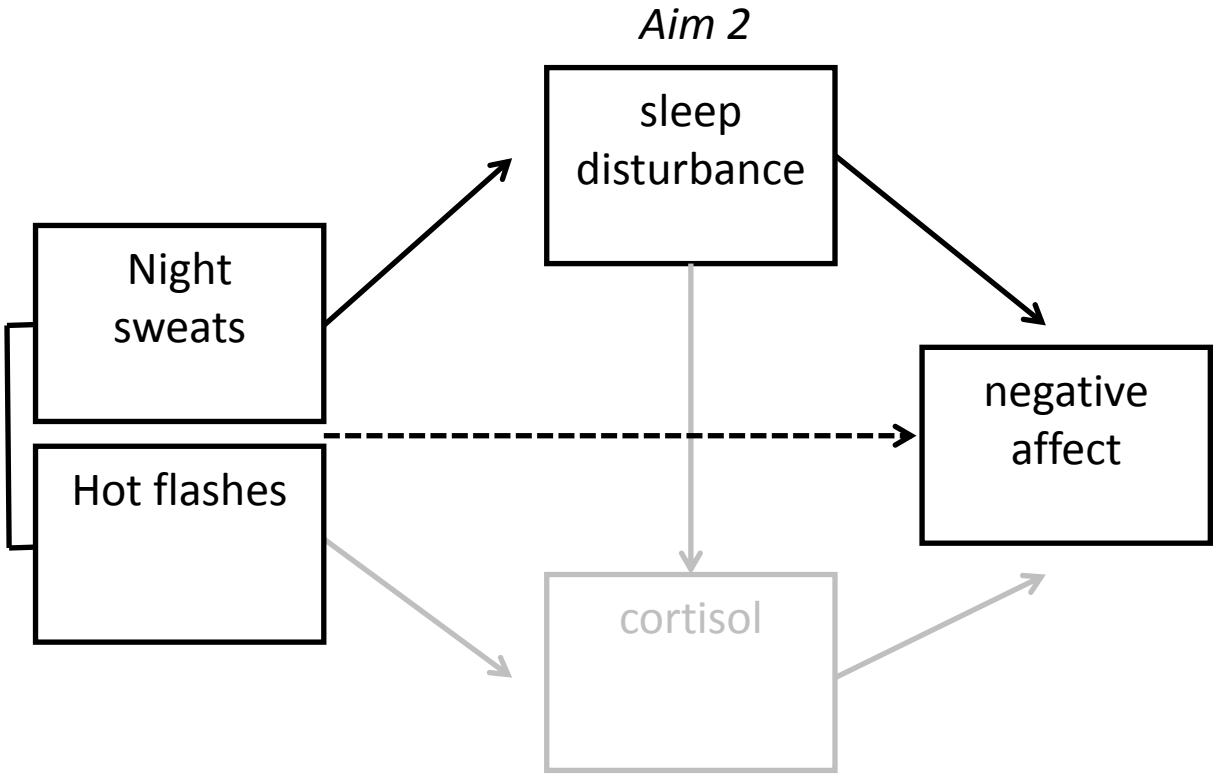

Figure 3. Aim 2: Role of sleep disturbance in relationship between VMS and next-day negative affect.

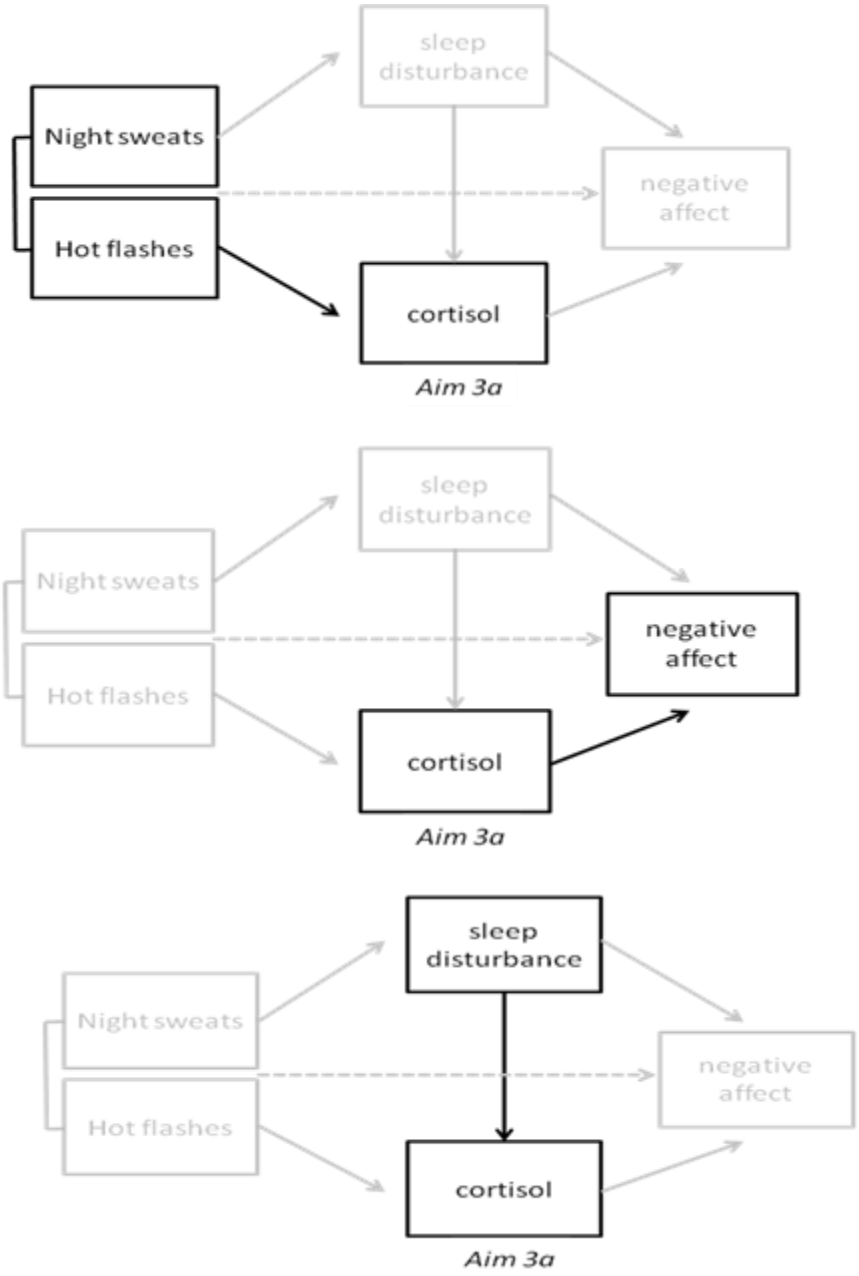

Figure 4. Aim 3a: Diurnal cortisol as a correlate of VMS, negative affect, and sleep parameters.

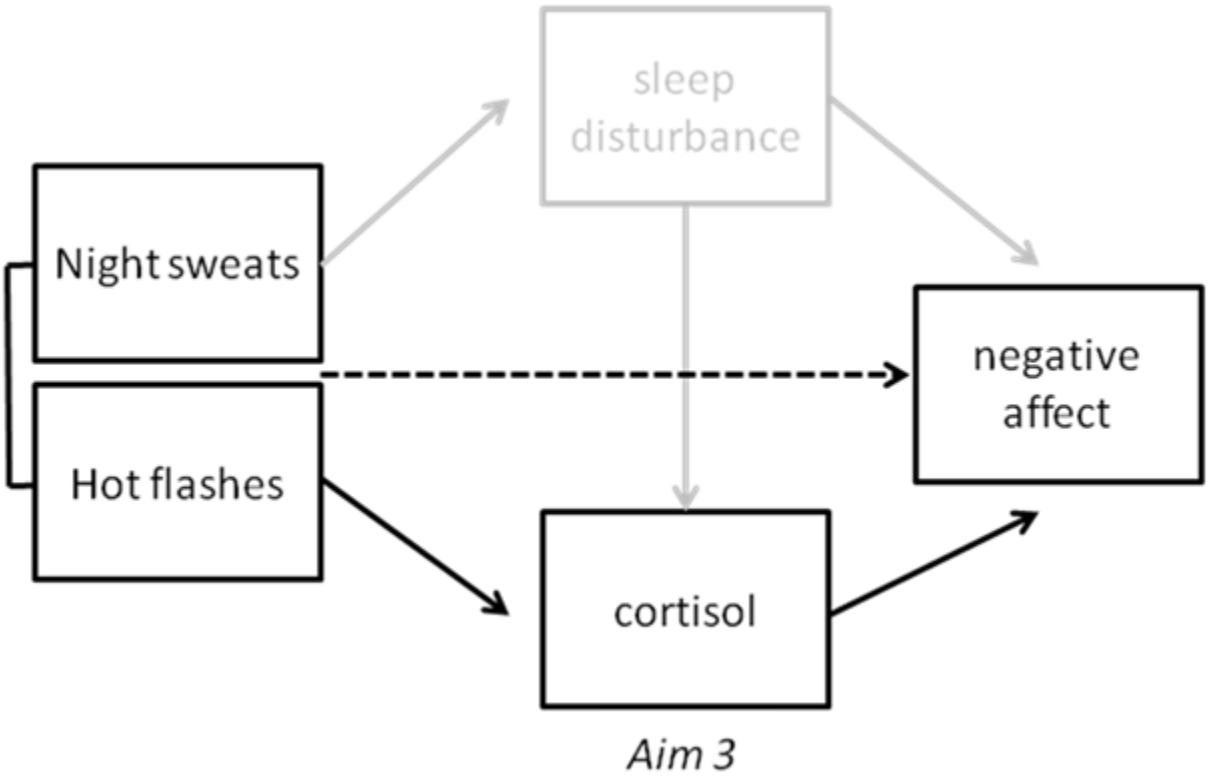

Figure 5. Aim 3b: Role of cortisol in relationship between VMS and negative affect.

Table 1. Characteristics of sample at baseline (n=53)

	Caucasian *(n=27, 50.98%)*	**African American** *(n=26, 49.02%)*	**Total** *(n=53)*
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
Age	53.44 (5.20)	53.15 (3.84)	53.31 (4.54)
Duration of vasomotor symptoms (years)	4.25 (4.40)	6.31 (6.80)	5.26 (5.74)
Body mass index (m/kg^2)*	27.36 (5.09)	31.78 (7.50)	29.53 (6.70)
Waist circumference (cm)*	89.63 (12.59)	102.96 (14.17)	96.03 (14.84)
Attitude Toward Aging and Menopause (Scale 1-3)±	2.46 (.38)	2.27(.45)	2.37 (.42)
Mini-IPIP (Scale 4-16)			
Extraversion	13.22 (3.36)	12.85 (3.65)	13.04 (3.47)
Agreeableness	15.96 (3.08)	15.56 (2.76)	15.77 (2.91)
Conscientiousness	16.00 (2.58)	15.36 (2.61)	15.68 (2.59)
Neuroticism	9.88 (3.41)	11.48 (3.55)	10.67 (3.54)
Intellect/Imagination	13.79 (3.32)	14.27 (2.92)	14.04 (3.10)
	n (%)	**n (%)**	**n (%)**
Menopausal status			
Perimenopausal	6 (22.20)	7 (26.90)	13 (24.50)
Postmenopausal	21 (77.80)	19 (73.10)	40 (75.50)
Education*			
High school degree or less	5 (18.50)	19 (73.10)	24 (45.30)
Some college	10 (37.00)	4 (15.40)	14 (26.40)
College degree or beyond	12 (44.40)	3 (11.50)	15 (28.30)
Self-Rated Health*			
Excellent/very good	19 (70.40)	10 (38.50)	29 (54.70)
Good/fair/poor	8 (29.60)	16 (61.50)	24 (45.30)
Body mass index (categorical)			
Normal weight (18.5-24.5)	9 (33.30)	4 (15.40)	13 (24.50)
Overweight (25-29.9)	12 (44.40)	10 (38.50)	22 (41.50)
Obese (>30)**	6 (22.20)	12 (46.20)	18 (34.00)

Mean comparisons by ANOVA, categorical by chi-square
*p<.05
**p<.09
± Higher scores are considered positive
Mini-IPIP: Mini-International Personality Item Pool

Table 2. Health behaviors of participants at baseline and during observation

	Caucasian (n=27, 49.02%)	**African American (n=26, 50.98%)**	**Total (n=53)**
	n (%)	**n (%)**	**n (%)**
Baseline visit questionnaires			
Habitual physical activity (IPAQ-SF)			
Low Physical Activity	9 (33.30)	7 (26.90)	16 (30.20)
Moderate Physical Activity¥	18 (66.70)	19 (73.10)	37 (69.80)
Electronic diaries			
Report alcohol intake on at least one day of observation			
Yes	12 (44.40)	13 (50.00)	25 (47.20)
No	15 (55.60)	13 (50.00)	28 (52.80)
Report caffeine intake on at least one day of observation			
Yes	22 (81.50%)	23 (88.50)	45 (84.90)
No	5 (18.50)	3 (11.50)	8 (15.10)
Report cigarette use on at least one day of observation**			
Yes	3 (11.10)	8 (30.80)	11 (20.80)
No	24 (88.90)	18 (69.20)	42 (79.20)
Report exercise on at least one day of observation			
Yes	24 (88.90)	21 (80.80)	45 (84.90)
No	3 (11.10)	5 (19.20)	8 (15.10)

Mean comparisons by ANOVA, categorical by chi-square
*p<.05
**p<.09
¥ IPAQ-SF: International Physical Activity Questionnaire, Short Form, completed at initial visit. Moderate physical activity defined as "a level of activity equivalent to half an hour of at least moderate-intensity physical activity on most days"

Table 3. Vasomotor symptoms at baseline and during observation

	Caucasian *(n=27, 50.98%)*	**African American** *(n=26, 49.02%)*	**Total** *(n=53)*
	n (%)	***n (%)***	***n (%)***
Telephone screen			
Number of vasomotor symptoms per 24 hours			
1-3	7 (25.90)	6 (23.10)	13 (24.50)
4-5	8 (29.60)	10 (38.50)	18 (34.00)
6+	12 (44.40)	10 (38.50)	22 (41.50)
Vasomotor symptom severity*			
Mild/moderate	24 (88.90)	12 (46.20)	36 (67.90)
Severe	3 (11.10)	14 (53.80)	17 (32.10)
Vasomotor symptom bother			
Not at all/very little	5 (18.50)	3 (11.50)	8 (15.10)
Moderately/a lot	22 (81.50)	23 (88.50)	45 (84.90)
Baseline questionnaires	***mean (SD)***	***mean (SD)***	***mean (SD)***
Hot Flash Daily Interference Score Mean (Scale 0-10)	3.42 (2.33)	4.67 (2.97)	4.03 (2.71)
Electronic diaries	***n (%)***	***n (%)***	***n (%)***
Number of vasomotor symptoms per 24 hours			
<1-3	6 (22.30)	4 (15.40)	10 (18.90)
4-5	3 (11.10)	5 (19.20)	8 (15.10)
6+	18 (66.70)	17 (65.40)	35 (66.00)
Vasomotor symptom severity			
Mild/moderate	26 (96.30)	25 (96.20)	51 (96.20)
Severe	1 (3.70)	1 (3.80)	2 (3.80)
Vasomotor symptom bother			
Not at all/very little	12 (44.40)	10 (38.50)	22 (41.50)
Moderately/a lot	15 (55.60)	16 (61.50)	31 (58.50)
Number of daytime hot flashes			
<1-3	2 (7.40)	9 (34.60)	11 (20.80)
4-5	7 (25.90)	3 (11.50)	10 (18.90)
6+	18 (66.70)	14 (53.80)	32 (60.40)
Hot flash rate (number per hour awake)	.34 (.29)	.53 (.48)	.44 (.41)
Hot flash severity			
Mild/moderate	26 (96.30)	25 (96.20)	51 (96.20)
Severe	1 (3.70)	1 (3.80)	2 (3.80)
Hot flash bother			
Not at all/very little	11 (40.70)	8 (30.80)	19 (35.80)

Moderately/a lot	16 (59.30)	18 (69.20)	34 (64.20)
Number of night sweats per night			
<1-3	8 (29.60)	9 (34.60)	17 (32.10)
4-5	1 (3.70)	5 (19.20)	6 (11.30)
6+	18 (66.70)	12 (46.20)	30 (56.60)
Night sweat rate (number per hour asleep)*	.24 (.19)	.44 (.32)	.34 (.28)
Night sweats severity			
Mild/moderate	26 (96.30)	23 (88.50)	49 (92.50)
Severe	1 (3.70)	3 (11.50)	4 (7.50)
Night sweats bother			
Not at all/very little	12 (44.40)	14 (53.80)	26 (49.10)
Moderately/a lot	15 (55.60)	12 (46.20)	27 (50.90)
Actigraphy event marker press	***n (%)***	***n (%)***	***n (%)***
Number of vasomotor symptoms per 24 hrs**			
1-3	4 (14.28)	6 (24.00)	10 (18.87)
4-5	5 (17.86)	0 (0.00)	5 (9.43)
6+	19 (67.86)	19 (76.00)	38 (71.70)

Mean comparisons by ANOVA, categorical by chi-square
*p<.05
**p<.09

Table 4. Negative affect at baseline and during observation.

	Caucasian *(n=27, 50.98%)*	**African American** *(n=26, 49.02%)*	**Total** *(n=53)*
	mean (SD)	***mean (SD)***	***mean (SD)***
Baseline visit questionnaires			
Spielberger State Anxiety (20-80)	31.35 (10.64)	32.00 (9.17)	31.63 (9.92)
Spielberger Trait Anxiety (20-80)	34.85 (10.20)	38.30 (7.91)	36.45 (9.34)
Anxiety Sensitivity Score (0-72)	17.78 (8.60)	20.77 (12.54)	19.25 (10.72)
CES-D (0-60, clinical cut-off: 16)	12.24 (9.31)	14.88 (7.95)	13.53 (8.68)
	n (%)	***n (%)***	***n (%)***
CES-D (categorical)			
Above clinical cut-off (16+)	12 (44.40)	13 (50.00)	28 (52.80)
Below clinical cut-off (<16)	15 (55.60)	13 (50.00)	25 (47.20)
	mean (SD)	***mean (SD)***	***mean (SD)***
Electronic diaries			
PANAS negative affect scale (10-50)	11.49 (3.50)	12.75 (3.89)	12.10 (3.71)
PANAS sadness subscale (5-25)	6.03 (3.00)	7.12 (3.09)	6.55 (3.06)
PANAS positive affect subscale* (10-50)	22.21 (7.22)	27.29 (6.21)	24.70 (7.18)
Gibson negative affect measure (3-15)	3.99 (.57)	4.62 (1.49)	4.30 (1.55)

Mean comparisons by ANOVA, categorical by chi-square
*p<.05
**p<.09
CES-D: Center for Epidemiologic Studies Depression Scale
PANAS: Positive and Negative Affect Schedule
Gibson negative affect measure: Items "mood swings, sensitive, irritable, anxious, difficulty concentrating, forgetful, blue" to mirror Gibson, 2011

Table 5. Sleep parameters at baseline and during observation

	Caucasian *(n=27, 50.98%)*	**African American** *(n=26, 49.02%)*	**Total** *(n=53)*
	mean (SD)	***mean (SD)***	***mean (SD)***
Baseline visit questionnaires: PSQI			
Sleep duration*	6.66 (1.23)	5.79 (1.48)	6.23 (1.41)
Sleep efficiency (%)	82.82 (14.25)	76.86 (32.53)	79.96 (24.71)
Overall sleep quality (0-3)£	1.26 (.66)	1.54 (.76)	1.40 (.72)
Total score* (Cut-off for poor sleep: >5)	6.96 (3.40)	9.36 (0.75)	8.06 (3.63)
	n (%)	***n (%)***	***n (%)***
Have a bed partner*			
Yes	17 (63.00)	8 (30.80)	25 (47.20)
No	10 (37.00)	18 (69.20)	28 (52.80)
Sleep duration*			
7+ hours	16 (59.30)	7 (26.90)	23 (43.40)
<7 hours	11 (40.70)	19 (73.10)	30 (56.60)
Overall sleep quality			
Best/good)	19 (70.40)	14 (53.80)	33 (62.30)
Fair/poor	8 (29.60)	12 (46.20)	20 (37.70)
Total score**			
Good sleep quality (≤5)	11 (40.70)	4 (15.40)	15 (28.30)
Poor sleep quality (>5)	16 (59.30)	22 (84.60)	38 (71.70)
	mean (SD)	***mean (SD)***	***mean (SD)***
Actigraphy			
Sleep duration (hours)*	6.02 (.91)	5.21 (.92)	5.63 (.98)
Sleep efficiency (%)*	80.23 (7.40)	71.18 (13.09)	75.95 (11.24)
Minutes of wake after sleep onset*	53.18 (21.50)	74.67 (45.67)	63.57 (36.10)
	mean (SD)	***mean (SD)***	***mean (SD)***
Electronic diaries			
"Feel rested this morning?" (1-6)	3.85 (.86)	4.01 (1.01)	3.93 (.93)
"Good night's sleep?" (1-6)	3.75 (.80)	3.74 (1.04)	3.75 (.92)
Number of naps/day during observation	.45 (.60)	.53 (1.02)	.49 (.82)
Total number of naps during week	2.59 (3.00)	2.52 (2.77)	2.56 (2.89)

Mean comparisons by ANOVA, categorical by chi-square
*p<.05
**p<.09
£ Scores range from 0 (best) to 3 (worst)
PSQI: Pittsburgh Sleep Quality Index

Table 6. Cortisol values over the observed period

	Caucasian *(n=27, 50.98%)*	**African American** *(n=26, 49.02%)*	**Total** *(n=53)*
	mean (SD)	***mean (SD)***	***mean (SD)***
Salivary cortisol			
Morning values (nmol/l)	17.62 (7.56)	15.72 (7.47)	16.82 (7.50)
Bedtime values (nmol/l)*	2.64 (2.55)	5.75 (4.93)	3.96 (4.00)
Diurnal slope**	-1.16 (.74)	-.70 (.80)	-.97 (.79)
Daily cortisol exposure (nmol/l)	10.19 (4.30)	10.84 (4.80)	10.46 (4.46)
Hair cortisol			
Three month value (nmol/l)	2.50 (5.43)	5.41 (5.59)	3.50 (5.58)*
Daily exposure			
Daily morning + bedtime average (nmol/l)	10.19 (4.30)	10.84 (4.80)	10.46 (4.46)

Mean comparisons by ANOVA

Though non-transformed means shown in this table, analyses were conducted using log-transformed values of both salivary and hair cortisol. Diurnal slope calculated from log-transformed salivary cortisol values (nmol/l), adjusted by self-reported time between bedtime and morning collection.

Morning and bedtime values calculated from all tubes labeled morning or bedtime, regardless of study day. Diurnal slope and overall daily cortisol exposure calculated only from tubes labeled morning and bedtime on three consecutive days. All calculations exclude original (non-logged) values <.3 nmol/l, >60 nmol/l, and samples unlabeled as to time of collection.

Table 7. Specific Aim 1A: Vasomotor Symptoms and Same Day Negative Affect

	PANAS Negative Affect†	**PANAS Sadness**	**Gibson Negative Affect£**
	B, p-value	***B, p-value***	***B, p-value***
Vasomotor Symptom Bother	1.46, <0.001	0.46, 0.04	0.44, <0.01
Daytime Hot Flash Bother	1.46, <0.001	0.46, 0.04	0.44, <0.01

Analyses conducted by multilevel hierarchical linear regression, using HLM 7.1 software.

†Exploratory moderation analyses suggest that these relationships are strongest among African American participants (trend for frequency*race, interaction for bother*race p<.01).
£Exploratory moderation analyses suggest that the relationship between vasomotor symptom bother and negative affect is strongest among African American participants (interaction for vasomotor symptom and daily hot flash bother*race p=.04).

Model adjusted by age, menopausal status, race/ethnicity, body mass index, educational attainment, self-rated health, time of day of diary reporting, and diary-reported health behaviors (exercise, caffeine intake, alcohol use, cigarette use), selected *a priori*. Only those predictors significantly associated with the outcome (p<.05) in both unadjusted and fully adjusted models shown.

Same day negative affect measured by Positive and Negative Affect Schedule: Negative Affect Subscale; Positive and Negative Affect Schedule: Sadness Subscale; Gibson Negative Affect (items "mood swings, sensitive, irritable, anxious, difficulty concentrating, forgetful, blue" to mirror Gibson, 2011).

Table 8. Specific Aim 1B: Vasomotor Symptoms and Next Day Negative Affect

	PANAS Negative Affect€	**Gibson Negative Affect**
	B, p-value	***B, p-value***
Hot Flash Frequency (previous day)	0.18, 0.046	
Night Sweat Severity (previous night)	0.80, 0.02	0.62, <0.01
Night Sweat Bother (previous night)	0.61, 0.02	0.35, 0.03

Analyses conducted by multilevel hierarchical linear regression, using HLM 7.1 software.

Model adjusted by age, menopausal status, race/ethnicity, body mass index, educational attainment, self-rated health, time of day of diary reporting, and diary-reported health behaviors (exercise, caffeine intake, alcohol use, cigarette use), selected *a priori*. Only those predictors significantly associated with the outcome ($p<.05$) in both unadjusted and fully adjusted models shown.

Same day negative affect measured by Positive and Negative Affect Schedule: Negative Affect Subscale; Gibson Negative Affect (items "mood swings, sensitive, irritable, anxious, difficulty concentrating, forgetful, blue" to mirror Gibson, 2011).

€When next day hot flash frequency is entered as the outcome in an otherwise equivalent model, no relationship is seen between previous day negative affect and next day hot flash frequency (*B*=0.01, p=0.33).

Table 9. Specific Aim 2: Role of Sleep Parameters in Relationship between Vasomotor Symptoms and Next Day Negative Affect

	PANAS Negative Affect				**Gibson Negative Affect**			
	No sleep in model	**Sleep duration in model**	**Sleep efficiency in model**	**WASO in model**	**No sleep in model**	**Sleep duration in model**	**Sleep efficiency in model**	**WASO in model**
	B, p-value	***B, p-value***	***B, p-value***	***B, p-value***	***B, p-value***	***B, p-value***	***B, p-value***	***B, p-value***
Night sweat severity	.78, 0.01	.86, 0.01	.82, 0.02	.85, 0.01	.48, <.01	.51, <.01	.48, <.01	.53, <.01
Night sweat bother					.29, 0.03	.30, 0.03	.29, 0.04	.32, .02

Analyses conducted by multilevel hierarchical linear regression, using HLM 7.1 software.

Model adjusted by age, menopausal status, race/ethnicity, body mass index, educational attainment, self-rated health, time of day of diary reporting, and diary-reported health behaviors (exercise, caffeine intake, alcohol use, cigarette use), selected *a priori*. Only those predictors significantly associated with the outcome ($p<.05$) in both unadjusted and fully adjusted models shown.

Same day negative affect measured by Positive and Negative Affect Schedule: Negative Affect Subscale; Positive and Negative Affect Schedule: Sadness Subscale; Gibson Negative Affect (items "mood swings, sensitive, irritable, anxious, difficulty concentrating, forgetful, blue" to mirror Gibson, 2011).

No other sleep variables exhibited significant associations with the measures of daily negative affect in base models, and so were not further analyzed in fully adjusted or mediational models.

Table 10. Specific Aim 3a: Relationship between Cortisol and Negative Affect

	PANAS Negative Affect ***B, p-value***	**PANAS Sadness** ***B, p-value***
Cortisol slope	.68, .01	.43, <.001
Mean daily cortisol		0.07, 0.03

Analyses conducted by multilevel hierarchical linear regression, using HLM 7.1 software.

Model adjusted by age, menopausal status, race/ethnicity, body mass index, educational attainment, self-rated health, time of day of diary reporting, and diary-reported health behaviors (exercise, caffeine intake, alcohol use, cigarette use), selected *a priori*. Only those predictors significantly associated with the outcome ($p<.05$) in both unadjusted and fully adjusted models shown.

Table 11. Specific Aim 3a: Relationship between Cortisol and Vasomotor Symptoms

	Vasomotor Symptom Severity *B, p-value*	**Daytime Hot Flash Frequency** *B, p-value*	**Daytime Hot Flash Severity†** *B, p-value*	**Daytime Hot Flash Bother** *B, p-value*
Cortisol slope	.06, 0.02		.09, 0.03	.10, <.01
Hair cortisol		0.05, 0.01		

Cortisol slope analyses conducted by multilevel hierarchical linear regression, using HLM 7.1 software; hair cortisol analyses conducted by linear regression, using SPSS 20.0 software.

†Exploratory moderation analyses by race/ethnicity suggest that the relationship between a flatter cortisol slope and daytime hot flash severity may be stronger among the African American participants (interaction *B* .11, p=.09, non-significant trend). No other differences in relationships by race/ethnicity were seen.

Model adjusted by age, menopausal status, race/ethnicity, body mass index, educational attainment, self-rated health, time of day of diary reporting, and diary-reported health behaviors (exercise, caffeine intake, alcohol use, cigarette use), selected *a priori*. Only those predictors significantly associated with the outcome (p<.07) in both unadjusted and fully adjusted models shown.

Table 12. Specific Aim 3a: Relationship between Cortisol and Sleep Parameters

	Sleep Duration (previous night)	**Sleep Efficiency (previous night)**	**WASO (previous night)**	**Sleep Duration (following night)**
	B, p-value	***B, p-value***		***B, p-value***
Cortisol slope	-.38, <.01		-7.62, 0.02	.49, 0.04
Mean daily cortisol		-.004, 0.03	2.68, 0.02	

Analyses conducted by multilevel hierarchical linear regression, using HLM 7.1 software.

Model adjusted by age, menopausal status, race/ethnicity, body mass index, educational attainment, self-rated health, time of day of diary reporting, and diary-reported health behaviors (exercise, caffeine intake, alcohol use, cigarette use), selected *a priori*. Only those predictors significantly associated with the outcome ($p<.05$) in both unadjusted and fully adjusted models shown.

Table 13. Specific Aim 3b: Diurnal Cortisol Slope as a Mediator Linking Vasomotor Symptoms with Negative Affect

	PANAS Negative Affect		Gibson Negative Affect	
	No cortisol	**Cortisol in model**	**No cortisol**	**Cortisol in model**
	B, p-value	***B, p-value***	***B, p-value***	***B, p-value***
Daytime hot flash frequency			-.31, 0.04	-.33, .045
Daytime hot flash severity ±	2.28, <.01	2.16, <.01	0.83, <.01	0.61, .048
Daytime hot flash bother π	1.73, <.01	1.75, <.01	.49, 0.04	.28, 0.17

Analyses conducted by multilevel hierarchical linear regression, using HLM 7.1 software.

Model adjusted by age, menopausal status, race/ethnicity, body mass index, educational attainment, self-rated health, time of day of diary reporting, and diary-reported health behaviors (exercise, caffeine intake, alcohol use, cigarette use), selected *a priori*. Only those predictors significantly associated with the outcome ($p<.05$) in both unadjusted and fully adjusted models shown.

±When cortisol slope replaced with mean daily cortisol exposure in otherwise equivalent models, B=0.10, p=0.08 (PANAS NA), B=0.02, p=0.13 (Gibson NA).

πWhen cortisol slope replaced with mean daily cortisol exposure in otherwise equivalent models, B=0.10, p=0.08 (PANAS NA), B=0.02, p=0.15 (Gibson NA).

Table 14. Correlation Matrix of Key Variables: Negative Affect

Negative Affect			
	PANAS Negative Affect (n=53)	**PANAS Sadness (n=53)**	**Gibson Negative Affect (n=53)**
Covariates			
Age (n=53)	-.33*	-.24	-.29*
BMI (n=53)	.09	.17	.07
Menopausal status (n=53)	-.24	-.27	-.25
Race/ethnicity (n=53)	.17	.17	.20
Education (n=53)	-.31*	-.26	-.36*
Self-rated health (n=53)	-.16	-.24	-.24
Report exercise in diary (n=39)	.31	.20	.25
Report caffeine intake in diary (n=39)	.24	.24	.15
Report alcohol use in diary(n=39)	.12	.06	.23
Report cigarette use in diary (n=39)	.53*	.50*	.53*
Predictor Variables			
Vasomotor Symptom Frequency (n=53)	.23	.08	.10
Vasomotor Symptom Severity (n=53)	.36*	.21	.31*
Vasomotor Symptom Bother (n=53)	.34*	.21	.25
Diurnal Cortisol Slope (n=44)	-.23	-.11	-.17
Overall Mean Daily Cortisol Exposure (n=44)	-.36*	-.43*	-.24
Hair Cortisol (n=35)	.16	.26	.12
Sleep Duration (n=52)	-.04	-.14	-.16
Sleep Efficiency (n=52)	-.21	-.26	-.31*
Sleep After Wake Onset (n=52)	.19	.21	.23

*p<.05

Referents in categorical variables: Race/ethnicity: Caucasian; Menopausal status: Perimenopausal; Education: High school or less; Self-rated health: Fair/poor; Health behaviors (exercise, caffeine, alcohol, cigarettes): No reported use over observed period; Vasomotor Symptom Severity: Mild; Vasomotor Symptom Bother: Not at all.

Table 15. Correlation Matrix of Key Variables: Vasomotor Symptoms

Vasomotor Symptoms			
	Vasomotor Symptom Frequency (n=53)	**Vasomotor Symptom Severity (n=53)**	**Vasomotor Symptom Bother (n=53)**
Covariates			
Age (n=53)	-.19	-.06	.02
BMI (n=53)	.14	.34*	.27*
Menopausal status (n=53)	-.17	-.04	-.03
Race/ethnicity (n=53)	.20	.16	.09
Education (n=53)	-.18	-.13	-.08
Self-rated health (n=53)	-.14	-.20	-.17
Report exercise in diary (n=39)	.25	-.05	.00
Report caffeine intake in diary (n=39)	-.21	.08	.06
Report alcohol use in diary (n=39)	.07	-.03	-.08
Report cigarette use in diary (n=39)	.22	.03	.02
Predictor Variables			
Diurnal Cortisol Slope (n=44)	.09	.06	.01
Overall Mean Daily Cortisol Exposure (n=44)	.03	-.05	-.01
Hair Cortisol (n=35)	.36*	.11	.00
Sleep Duration (n=52)	.12	-.04	-.07
Sleep Efficiency (n=52)	-.10	-.29*	-.23
Sleep After Wake Onset (n=52)	.26*	.21	.16
PANAS Negative Affect (n=53)	.23	.36*	.34*
PANAS Sadness (n=53)	.08	.21	.21
Gibson Negative Affect (n=53)	.10	.31*	.25

*p<.05

Referents in categorical variables: Race/ethnicity: Caucasian; Menopausal status: Perimenopausal; Education: High school or less; Self-rated health: Fair/poor; Health behaviors (exercise, caffeine, alcohol, cigarettes): No reported use over observed period; Vasomotor Symptom Severity: Mild; Vasomotor Symptom Bother: Not at all.

Table 16. Correlation Matrix of Key Variables: Cortisol

Cortisol			
	Diurnal Cortisol Slope (n=44)	**Overall Mean Daily Cortisol Exposure (n=44)**	**Hair Cortisol (n=39)**
Covariates			
Age (n=53)	.02	.11	-.06
BMI (n=53)	.20	-.14	.24
Menopausal status (n=53)	.17	.09	-.32
Race/ethnicity (n=53)	-.01	.07	.45*
Education (n=53)	-.33*	.00	-.20
Self-rated health (n=53)	-.20	-.07	-.19
Report exercise in diary (n=39)	.16	-.30	.26
Report caffeine intake in diary (n=39)	-.27	.05	.06
Report alcohol use in diary (n=39)	-.01	-.19	-.23
Report cigarette use in diary (n=39)	.08	-.12	.47*
Predictor Variables			
Sleep Duration (n=52)	-.12	.14	.30
Sleep Efficiency (n=52)	-.01	-.01	-.35*
Sleep After Wake Onset (n=52)	.11	.22	.57*
PANAS Negative Affect (n=53)	-.23	-.36*	.16
PANAS Sadness (n=53)	-.11	-.43*	.26
Gibson Negative Affect (n=53)	-.17	-.24	.12
VMS Frequency (n=53)	-.10	.03	.36*
VMS Severity (n=53)	.06	-.05	.11
VMS Bother (n=53)	.01	-.01	.00

*p<.05

Referents in categorical variables: Race/ethnicity: Caucasian; Menopausal status: Perimenopausal; Education: High school or less; Self-rated health: Fair/poor; Health behaviors (exercise, caffeine, alcohol, cigarettes): No reported use over observed period; Vasomotor Symptom Severity: Mild; Vasomotor Symptom Bother: Not at all.

Table 17. Correlation Matrix of Key Variables: Sleep

Sleep			
	Sleep Duration (n=52)	**Sleep Efficiency (n=52)**	**Wake After Sleep Onset (n=52)**
Age (n=53)	-.25	.06	-.12
BMI (n=53)	-.16	-.16	.15
Menopausal status (n=53)	-.20	.14	-.20
Race/ethnicity (n=53)	-.06	-.30*	.32*
Education (n=53)	.12	.25	-.10
Self-rated health (n=53)	-.08	.38*	-.50*
Report exercise in diary (n=39)	-.12	-.36*	.42*
Report caffeine intake in diary (n=39)	-.12	.04	-.17
Report alcohol use in diary (n=39)	-.11	.03	.03
Report cigarette use in diary (n=39)	-.12	-.03	-.15
Predictor Variables			
PANAS Negative Affect (n=53)	-.04	-.21	.19
PANAS Sadness (n=53)	-.14	-.26	.21
Gibson Negative Affect (n=53)	-.16	-.31*	.23
VMS Frequency (n=53)	.12	-.10	.26
VMS Severity (n=53)	-.14	-.26	.32*
VMS Bother (n=53)	-.07	-.18	.28*
Diurnal Cortisol Slope (n=44)	-.18	.16	.04
Overall Mean Daily Cortisol Exposure (n=44)	.14	-.01	.22
Hair Cortisol (n=35)	.30	-.35*	.57*

*p<.05

Referents in categorical variables: Race/ethnicity: Caucasian; Menopausal status: Perimenopausal; Education: High school or less; Self-rated health: Fair/poor; Health behaviors (exercise, caffeine, alcohol, cigarettes): No reported use over observed period; Vasomotor Symptom Severity: Mild; Vasomotor Symptom Bother: Not at all.

APPENDIX A

KEY VARIABLE CATEGORIES FOR STATISTICAL ANALYSIS

VMS: Occurrence (yes/no), frequency (number), rate (number reported within observation period, based on diary timestamps), severity (mild: sensation of heat without sweating; moderate: sensation of heat with sweating, able to continue activity; severe: sensation of heat with sweating, causing cessation of activity), and bother (not at all, very little, moderately, a lot).

NS: Night sweats, reported in morning diaries and with event marker button press overnight.

Negative affect: PANAS negative affect scale, positive affect scale, sadness subscale; negative affect measure comparable to Gibson et al. (Gibson et al., 2011).

Sleep: Actigraphy-derived sleep duration, sleep efficiency, and wake after sleep onset.

Cortisol: Diurnal slope calculated from awakening value subtracted from bedtime value, adjusted by day length; cortisol exposure calculated as the average of the morning and bedtime value for each participant, each day; and an overall value assessed from hair samples.

APPENDIX B

STATISTICAL ANALYSIS EQUATIONS

B.1 SPECIFIC AIM 1

Specific Aim 1: Examine the contribution of VMS to within-day and next-day negative affect.

Using three-level hierarchical linear models, within day analyses were conducted with observations (level 1) nested with days (level 2), nested within participants (level 3), negative affect was regressed on VMS from the same day (1) and the previous day (2). Additionally, negative affect was regressed on night sweats from the previous night reporting in morning diaries (3).

Equations:

1) Negative affect$_{ijk}$= γ_{000} + γ_{100}VMS$_{ijk}$* + r_0 + u_{00} + e
2) Negative affect$_{ijk}$ = γ_{000} + γ_{100}lagVMS$_{ijk}$*+ r_0 + u_{00} + e
3) Negative affect$_{ijk}$ = γ_{000} + γ_{010}NS$_{jk}$* + r_0 + u_{00} + e

Variables:

Negative affect$_{ijk}$: Negative affect of observation *i* in day *j* of participant *k*

γ_{000}: Negative affect grand mean

γ_{100}VMS$_{ijk}$: Main effect of same-day VMS on negative affect

γ_{100}lagVMS$_{ijk}$: Main effect of previous day VMS on negative affect

γ_{010}NS$_{ijk}$: Main effect of previous night's night sweats on negative affect

r_0: Random day effect (deviation of day *j*'s mean from the participant mean)

u_{00}: Random participant effect (deviation of participant *k*'s mean from the grand mean)

e: Random observation effect (deviation of observation *ijk*'s value from the day mean)

B.2 SPECIFIC AIM 2

Specific Aim 2: Examine sleep as a mediator of the association between VMS and next-day negative affect.

In order to test mediation in multilevel models, variables in the mediational chain may affect a variable at the same or lower levels, but not higher levels (Kenny, Korchmaros, & Bolger, 2003). As VMS and negative affect are measured at level 1 (multiple timepoints within a day), and sleep is measured at level 2 (one variable for each sleep parameter per day), it was necessary to aggregate VMS and negative affect within participants, within each day. Mediation was then tested with two-level hierarchical models using the Krull-MacKinnon method (Krull & MacKinnon, 1999), with mean daily observations (level 1) nested within women (level 2) to assess observations within women. In the first step (1), negative affect was predicted from

previous day VMS with two-level hierarchical linear regression. In the second step (2), sleep from the night prior to the negative affect measurement was included in the regression model in order to estimate negative affect from both VMS and sleep (A). The difference between the estimates of the VMS variable in the first and second equation estimated the extent to which sleep accounts for the relationship between VMS and next day negative affect. Analyses were repeated with self-reported nocturnal VMS from morning diaries from the same day that negative affect was reported in place of previous day VMS (B).

Equations:

A)

1) $\text{Negative affect}_{jk} = \zeta_{00} + \zeta_{10}\text{lagVMS}_{jk}* + r_0 + r_1\text{lagVMS}_{jk} + e$
2) $\text{Negative affect}_{jk} = \zeta_{00} + \zeta_{10}\text{lagVMS}_{jk} + \zeta_{20}\text{sleep}_{jk}* + r_0 + r_1\text{lagVMS}_{jk} + r_2\text{sleep}_{jk} + e$

B)

1) $\text{Negative affect}_{jk} = \zeta_{00} + \zeta_{10}\text{NS}_{jk} + r_0 + r_1\text{NS}_{jk} + e$
2) $\text{Negative affect}_{jk} = \zeta_{00} + \zeta_{10}\text{NS}_{jk} + \zeta_{20}\text{sleep}_{jk}* + r_0 + r_1\text{NS}_{jk} + r_2\text{sleep}_{jk} + e$

Variables:

$\text{Negative affect}_{jk}$: Aggregate negative affect from day *j* of participant *k*

ζ_{00}: Negative affect grand mean

$\zeta_{10}\text{lagVMS}_{jk}$: Main effect of aggregate previous day VMS on aggregate negative affect

$\zeta_{20}\text{sleep}_{jk}$: Main effect of previous night's sleep on aggregate negative affect

r_0: Random participant effect (deviation of participant *k*'s mean from the grand mean)

$r_1\text{lagVMS}_{jk}$: Random VMS effect

$r_1\text{NS}_{jk}$: Random night sweats effect

$r_2\text{sleep}_{jk}$: Random sleep effect

e: Random day effect (deviation of day *j*'s mean from the participant mean)

B.3 SPECIFIC AIM 3A

Specific Aim 3a: Examine diurnal cortisol slope and hair cortisol as physiologic measures associated with VMS, sleep parameters, and negative affect

Cortisol, sleep, VMS, and negative affect data were collected at different levels, with salivary cortisol collected on only 3 of the 7 days comprising level 2 and hair cortisol measured from a single sample to assess overall (past three month) exposure. As HLM is not robust to missing data for primary predictors at level 2 in either 2 or 3 levels models, this altered the appropriate analytic approaches to testing associations, and limited ability to examine temporal relations between these factors over the observed period. These relationships were therefore examined in several ways, with one and two level data structure categorization. For diurnal cortisol, analysis of all variables was first restricted to the three days of cortisol collection (A), and aggregate measures resulting in one value for each variable per participant over the observed period (B). The latter was repeated with hair cortisol values in place of the aggregate mean diurnal slope value. The limitations of these approaches is acknowledged, as the restricted range of observations will reduce reliability and power, and aggregate measures may cloud inter- and intraindividual differences in key measures.

A) 1. Using two-level hierarchical linear modeling restricted to the three days of cortisol collection, diurnal cortisol slope at level 2 was separately modeled as a function of VMS (a), negative affect (b), and sleep parameters (c) from the night preceding and following sampling.

B) 1. Using linear regression models, aggregate measures of all observations per participant of VMS, diurnal cortisol slope, and sleep were examined. Diurnal cortisol slope was modeled separately as a function of VMS (a), negative affect (b), and sleep (c).

2. This same analysis will be repeated with the single overall cortisol measure from hair sampling, such that hair cortisol values were modeled separately as a function of VMS (a), negative affect (b), and sleep (c).

Equations:

A1a) $\text{Cortisol}_{jk} = \zeta_{00 + \zeta 10}\text{VMS}_{jk}^{*} + r_0 + e$

A2b) $\text{Cortisol}_{jk} = \zeta_{00 +} \zeta_{10}\text{NA}_{jk}^{*} + r_0 + e$

A2c) $\text{Cortisol}_{jk} = \zeta_{00 +} \zeta_{10}\text{sleep}_{jk}^{*} + r_0 + e$

B1/2a) $\text{Cortisol} = B_0 + B_1\text{VMS}^{*} + e$

B1/2b) $\text{Cortisol} = B_0 + B_1\text{negative affect}^{*} + e$

B1/2c) $\text{Cortisol} = B_0 + B_1\text{sleep}^{*} + e$

Variables:

A1)

Cortisol_{jk}: Diurnal cortisol slope from day *j* of participant *k*

ζ_{00}: Diurnal cortisol slope grand mean

$\zeta_{10}\text{VMS}_{jk}$: Main effect of VMS on diurnal cortisol slope

$\zeta_{10}\text{NA}_{jk}$: Main effect of negative affect on diurnal cortisol slope

$\zeta_{10}\text{sleep}_{jk}$: Main effect of sleep on diurnal cortisol slope

r_0: Random participant effect (deviation of participant *k*'s mean from the grand mean)

e: Random day effect (deviation of day *j*'s mean from the participant mean)

B1)

Cortisol: Mean diurnal cortisol slope

B_0: intercept

B_1VMS: Main effect of mean VMS on diurnal cortisol slope

B_1NS: Main effect of mean night sweats on diurnal cortisol slope

B_1negative affect: Main effect of mean negative affect on diurnal cortisol slope

B_1sleep: Main effect of mean sleep on diurnal cortisol slope

e: error term

B2)

Cortisol: Hair cortisol value

B_0: intercept

B_1VMS: Main effect of mean VMS on hair cortisol value

B_1NS: Main effect of mean night sweats on hair cortisol value

B_1negative affect: Main effect of mean negative affect on hair cortisol value

B_1sleep: Main effect of mean sleep on hair cortisol value

e: error term

B.4 SPECIFIC AIM 3B

Specific Aim 3b: Examine diurnal cortisol as a mediator linking VMS with negative affect.

In order to test mediation in multilevel models, variables in the mediational chain may affect a variable at the same or lower levels, but not higher levels. Mediation was tested with

two-level hierarchical models using the Krull-MacKinnon method (Krull & MacKinnon, 1999) with data from the three days of cortisol collection (A), and with simple linear regression using the Sobel method (Sobel, 1982) with mean aggregate values of all parameters over the observed period (B).

A) In the first step (1), negative affect was predicted from VMS with two-level hierarchical linear regression. In the second step (2), diurnal cortisol slope was included in the regression model in order to estimate negative affect from both VMS and diurnal cortisol. The difference between the estimates of the negative affect slope variable in the first and second equation estimate the extent to which diurnal cortisol slope accounts for the relationship between VMS and negative affect.

B) In the first step (1), mean negative affect was predicted from mean VMS and mean diurnal cortisol slope with linear regression. In the second step (2), diurnal cortisol slope was predicted from mean VMS. The Sobel product of coefficients was calculated by multiplying the partial regression effect for diurnal cortisol slope predicting negative affect by the simple coefficient for VMS predicting diurnal cortisol slope (3). Bootstrapping methods were used to test for significance.

Equations:

A)

1. Negative affect$_{jk}$ = ζ_{00} + ζ_{10}VMS$_{jk}$* + r$_0$ + r$_1$VMS$_{jk}$ + e
2. Negative affect$_{jk}$ = ζ_{00} + ζ_{10}VMS$_{jk}$ + ζ_{20}cortisol$_{jk}$* + r$_0$ + r$_1$VMS$_{jk}$ + r$_2$cortisol$_{jk}$ + e

B)

1. Negative affect = B_0 + B_1VMS + B_2cortisol + e
2. Cortisol = B_0 + BVMS + e

3. $B_{indirect}$* = (B_2cortisol)(BVMS)

Variables:

A)

Negative affect$_{jk}$: Mean negative affect from day *j* of participant *k*

ζ_{00}: Negative affect grand mean

ζ_{10}VMS$_{jk}$: Main effect of mean VMS from day *j* of participant *k* on negative affect

ζ_{20}cortisol$_{jk}$: Main effect of diurnal cortisol slope from day *j* of participant *k* on negative affect

r_0: Random participant effect (deviation of participant *k*'s mean from the grand mean)

r_1VMS$_{jk}$: Random VMS effect

$r_1$cortisol$_{jk}$: Random cortisol effect

e: Random day effect (deviation of day *j*'s mean from the participant mean)

B)

Negative affect = Negative affect dependent variable

Cortisol = Diurnal cortisol slope dependent variable

B_0 = Dependent variable intercept

B_1VMS = Main effect of VMS on negative affect, controlling for diurnal cortisol slope

B_2cortisol = Main effect of diurnal cortisol slope on negative affect, controlling for VMS

BVMS = Main effect of VMS on diurnal cortisol slope

$B_{indirect}$ = Indirect effect of diurnal cortisol slope on negative affect

e = error term

APPENDIX C

ELECTRONIC DIARY TEMPLATES

Sample signaled-entry diary (each item appears on an individual screen; participants had verbal and written instructions about the scales utilized):

Since last entry,

How MANY hot flashes?

(Drop-down menu for #)

0, 1, … 5+

……..

What TIME last hot flash?

_____

……..

How SEVERE were they?

0/none 1/mild 2/moderate 3 /severe

……

How BOTHERSOME were they?

0/none 1/not at all 2/very little 3/moderately 4/a lot

……

How IRRITABLE do you feel right now?

1 2 3 4 5

(same format repeated for all individual mood items)

……

Are you alone?

Y N

……

Where are you?

1/at work 2/at home 3/in public

Sample self-initiated morning diary:

Time got into bed?

_____

AM PM

……..

Time tried to go to sleep?

_____

AM PM

……..

Time woke up?

_____

AM PM

……..

Any trouble sleeping?

NO YES

……..

This morning I feel rested:

NO! NO no yes YES YES!

……..

My sleep last night was very good:

NO! NO no yes YES YES!

……..

Overnight, how many hot flashes/since you went to bed?

_____

……..

What TIME last hot flash?

_____

AM PM

……..

How SEVERE were they?

0/none 1/mild 2/moderate 3 /severe

……

How BOTHERSOME were they?

0/none 1/not at all 2/very little 3/moderately 4/a lot

……

Sample self-initiated bedtime diary:

Since last entry,

How MANY hot flashes?

_____

……..

How SEVERE were they?

0/none 1/mild 2/moderate 3 /severe

……

How BOTHERSOME were they?

0/none 1/not at all 2/very little 3/moderately 4/a lot

……

How IRRITABLE do you feel right now?

1 2 3 4 5

(same format repeated for all individual mood items)

……

Did you EXERCISE today?

NO YES

(If yes):

Last time today?

MORNING AFTERNOON EVENING

……

Did you have CAFFEINE today?

NO YES

(If yes):

Last time today?

MORNING AFTERNOON EVENING

……

Did you drink ALCOHOL today?

NO YES

(If yes):

Last time today?

MORNING AFTERNOON EVENING

……

Did you smoke CIGARETTES today?

NO YES

(If yes):

Last time today?

MORNING AFTERNOON EVENING

……

Did you sleep during the daytime today?

NO YES

(If yes): How MANY times did you take a nap today?

_____

Nap times:

From _____ AM/PM to _____ AM/PM

From _____ AM/PM to _____ AM/PM

From _____ AM/PM to _____ AM/PM

From _____ AM/PM to _____ AM/PM

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