The Relationship between Vasomotor Symptoms and the Cortisol Awakening Response during the Menopause Transition

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Abstract

Vasomotor symptoms (VMS) – hot flashes and night sweats – are one of the most frequent symptoms of the menopause transition. While moderate to severe VMS have been associated with an increased risk for cardiovascular disease, the mechanisms underlying this relationship are not fully understood. One possibility may involve VMS’ link with dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, the body’s primary stress system, which releases the stress hormone cortisol. VMS have been related to an altered cortisol profile, including decreased morning cortisol, though the direction of the VMS-cortisol relationship is unknown. The current study aimed to determine the direction of this relationship. Of 82 healthy women ages 45-55 from the community, 13 women who were quite a bit or extremely bothered by VMS were included in the analysis, in the menopause transition. Once a week for 12 weeks, participants completed questionnaires on their hot flashes, mood, and sleep and provided two saliva samples for the measurement of cortisol: one immediately after waking up and one 30 minutes after waking up. A linear mixed model (PROC MIXED in SAS 4.3) indicated that although past-week CAR change was predictive of the number of severe VMS, past-week VMS change was a better predictive of the CAR. Past-week severe VMS change, total VMS change, and VMS score change was a statistically significant predictor of weekly morning cortisol. The current study indicates that treating the VMS directly rather than HPA axis dysregulation would better reduce the severity of the symptoms and reduce the HPA axis dysregulation.

Key words: cortisol, menopause transition, vasomotor symptoms
Acknowledgements

I would like to sincerely thank all the FEMM Study staff and volunteers for the hard work during the duration of this project. A special thank you to Laurie Sykes Tottenham, PhD for assisting our team with the assays. Without her guidance this study would not have run as smoothly. Finally, I would like to thank my supervisor, Jennifer L. Gordon, PhD. Without her dedication and guidance this manuscript would not have been possible. She has taught me so much and given me opportunities I have dreamed of. She has helped me grow not only as an academic but as a person.
Dedication

First and foremost, I must thank my parents. Their contribution exceeds the financial support they have given me. I thank them for every textbook, every class, every night I have had a loving home to go to, every word of encouragement, and every comforting hug. They have shaped me as a person and I owe much of my success to their love and support. Thank you to my friends who have been my second family throughout this process. I will always be grateful for their help during this process.
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The Relationship between Vasomotor Symptoms and the Cortisol Awakening Response during the Menopause Transition

The menopause transition (i.e., perimenopause) describes the transition from regular menstrual cycles to the complete cessation of menstruation, which typically begins in a woman’s mid-40s and lasts an average of five years (Burger et al., 2007). The menopause transition is defined by the Stages of Reproductive Aging Workshop (STRAW+10) and is separated into two categories: the early menopause transition and the late menopause transition (Harlow et al., 2012). A woman is in the early menopause transition when she experiences irregularity in her menstrual cycle, such that menses consistently occur seven days earlier or later than usual. The late menopause transition occurs when a woman misses at least two menstrual cycles or experiences at least 60 days without menstruation. Triggered by advancing age, the menopause transition is accompanied by several important hormonal changes, including an increase in estradiol fluctuation. On the one hand, estradiol levels can reach higher peaks than is typical; on the other hand, estradiol levels can drop to lower levels than would be expected in a typical menstrual cycle (Deecher & Dorries, 2007). This hormonal environment is believed to contribute to several physical symptoms that are commonly experienced in the menopause transition, including vasomotor symptoms (VMS; i.e., hot flashes and night sweats), sleep disturbances, and depressive symptoms (e.g., Arakane et al., 2011; Deecher, 2005; Freeman, 2015).

VMS affect 40-60% of women in the menopause transition (Deecher, 2005; Freeman & Sherif, 2007). During a hot flash, women have a subjective sensation of heat radiating the body (Deecher & Dorries, 2007). This sensation lasts approximately one to five minutes and can sometimes end in chills. Studies examining the physiological correlates of hot flashes have found that during hot flashes, heart rate increases, sweating occurs, and blood flow rises (Deecher,
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2005; Deecher & Dorries, 2007). Body temperature does, in fact, rise 0.7 degrees Celsius per minute during a hot flash (Germaine & Freedman, 1984). Although the exact mechanisms contributing to hot flashes are not well-known, withdrawal from estradiol is believed to be involved (Santoro, 2017). The anterior hypothalamus plays a major role in regulating body temperature and it has been theorized that withdrawal from estradiol may affect its ability to do so effectively (Deecher & Dorries, 2007).

The presence of VMS have been linked to an increased risk of several chronic illnesses, the strongest relationship being with cardiovascular disease (CVD). Studies have identified that women with VMS versus without VMS have more CVD events (Herber-Gast, Brown, & Mishra, 2015) and a poorer CVD risk profile (Gordon et al., 2016). In one meta-analytic review of 11 studies examining the relationship between VMS and CVD risk factors, five studies found VMS to be associated with higher systolic blood pressure and four studies found VMS to be linked with higher diastolic blood pressure (Franco et al., 2015). The same analysis reported that VMS were associated with an increased risk of hypertension, elevated cholesterol levels, and a higher BMI. While it is unknown why VMS are associated with CVD risk factors and an increased risk of CVD itself, increased activation of the hypothalamic-pituitary-adrenal (HPA) axis (the primary stress axis in humans) may help explain the relationship.

1.1 Vasomotor Symptoms and Hypothalamic-Pituitary-Adrenal Axis Dysregulation

The HPA axis is the body’s primary hormonal stress response system and is composed of the hypothalamus, the anterior pituitary gland, and the adrenal cortex (Frodl & O’Keane, 2013; Tsigos & Chrousos, 2002). Corticotropic releasing hormone is released in response to psychological stress (e.g., public speaking) or somatic stimuli (e.g., hunger) and regulates the secretion of adrenocorticotropic hormone (ACTH) by the anterior pituitary (Frodl & O’Keane,
ACTH then indirectly leads to the secretion of glucocorticoids, primarily cortisol, by the adrenal cortex (Tsigos & Chrousos, 2002). In a healthy individual, cortisol levels secreted by the HPA axis follow a predictable pattern throughout the day. Upon awakening, there is a 38-75% increase in cortisol, with levels peaking 20-30 minutes after individuals wake up (Fries, Dettenborn, & Kirschbaum, 2009). This response is known as the cortisol awakening response (CAR). After the CAR, cortisol concentrations decline dramatically a few hours after waking before following a slower, steady decline until the late evening when levels are at their lowest (Adam, Hawkley, Kudielka, & Cacioppo, 2006).

Research has predominately found that women suffering from more severe or more frequent VMS exhibit an altered daily cortisol pattern. One of the first studies linking VMS with cortisol was the Seattle Women’s Health Study, a longitudinal study of 169 women in the menopause transition or early postmenopause finding that an increase in cortisol levels from the early to late perimenopause were associated with an increase in VMS (Woods, Carr, Tao, Taylor, & Mitchell, 2006). Consistent with this finding is the second study by Gordon et al. (2016) of 186 perimenopausal and early postmenopausal women, finding that the extent to which participants reported being bothered by night sweats was positively associated with cortisol levels in the late afternoon. In a third study of 44 perimenopausal and postmenopausal women, hair cortisol, which provides a measure of total cortisol exposure over the previous three months, and diurnal salivary cortisol over a three-day period were measured (Gibson, Thurston, & Matthews, 2016). Results found that the number of reported hot flashes were associated with increased hair cortisol as well as a flatter salivary cortisol slope over the day; that is, lower morning cortisol and higher evening cortisol. A fourth study found that a within-person increase in hot flash frequency correlated with lower salivary cortisol concentrations 30 minutes after
awakening (Reed et al., 2016). In contrast to the above-described studies, however, one study found that in a sample of 40 participants, VMS were positively correlated with cortisol levels 15, 30, and 45 minutes upon awakening (Rubin, Drogos, Kapella, Geller, & Maki, 2014). An additional study found no relationship between VMS and cortisol levels (Gerber, Sievert, & Schwartz, 2017). This anomaly by Gerber et al. (2017) within the literature may have been due to the retrospective nature of the study. Participants were asked if they had experienced hot flashes within the last two weeks and saliva samples were measured to obtain diurnal cortisol for one day. The link between cortisol and VMS may be better understood through longitudinal studies that examine cortisol levels and hot flashes during the same time-period. Taken together, the literature to date suggests that, with two exceptions, VMS severity may be associated with lower morning cortisol but higher afternoon and evening cortisol, translating to overall increased cortisol output.

This link between VMS and altered cortisol release may help to explain why VMS is associated with an increased risk of CVD. Alterations in cortisol – specifically elevated concentrations – have been associated with CVD risk factors (Walker, 2007). For example, individuals taking high-dose prescription glucocorticoids (similar to cortisol) have been found to be at higher risk of CVD than individuals who do not take glucocorticoids (Wei, MacDonald, & Walker, 2004). Increased HPA axis activation during mental stressor tasks has been associated with coronary artery calcification, a predictor of coronary heart disease (Hamer, O’Donnell, Lahiri, & Steptoe, 2009). Higher cortisol concentrations have been related to plaque in the carotid arteries (Dekker et al., 2008), higher plasma glucose concentrations, and higher blood pressure, all of which are risk factors for CVD (Walker, 2007). There is therefore, reason to
believe that the link between VMS and cortisol may help to explain the relationship between VMS and CVD.

1.2 Rationale for the Current Study

Although several studies have observed a relationship between VMS and cortisol secretion, the direction of this relationship is currently unknown. One study of 18 participants experiencing severe hot flashes examined cortisol levels before, during, and after a hot flash (Meldrum et al., 1984). Results found that cortisol increased following the hot flash, peaking 15 minutes post-hot flash, suggesting that VMS may increase cortisol. Furthermore, VMS have been found to significantly predict cortisol levels during the transition into perimenopause (Woods, Mitchell, & Smith-DiJulio, 2009). However, it is also possible that suffering from repeated, severe hot flashes may indirectly increase cortisol levels given that VMS have been associated with depressive symptoms (Worsley, Bell, Kulkarni, & Davis, 2014) and is highly correlated with sleep disturbances (e.g., Arakane et al., 2011; Bursch, 2008). It has also been proposed that increased activation of the HPA axis may alter the hypothalamus’ regulation of body temperature, thereby increasing one’s susceptibility to experiencing VMS (Deecher & Dorries, 2007).

One promising approach to clarifying the direction of the relationship between VMS and HPA axis activation may be to examine the relationship between repeated measurements of VMS and cortisol within the same women over time. The current study included 13 perimenopausal women who were bothered by their hot flashes. Once per week for 12 weeks participants: recorded their VMS frequency and severity for 24 hours, collected two morning saliva samples for the measurement of the cortisol awakening response, and completed questionnaires about their previous night’s sleep and their depressive symptoms. The objectives of this study were to
investigate the following: a) the extent to which weekly changes in self-reported VMS are predictive of weekly CAR; b) the extent to which weekly changes in the CAR are predictive of weekly self-reported VMS, and c) the extent to which depressive symptoms and poor sleep mediate these two relationships. It was hypothesized that, although there will be a bidirectional relationship between VMS and the CAR, changes in VMS will be a stronger predictor of the CAR than changes in the CAR will be of VMS.

**Methods**

2.1 **Participants**

Data collected for this study are from the Fluctuating Estrogen and Menopausal Mood (FEMM) Study which is examining the effects of estradiol levels and depressive symptoms in women in the menopause transition. The FEMM study has been reviewed and approved by the University of Regina Ethics Board. Thirteen women between the ages of 45 and 55 years in the menopause transition according to STRAW+10 criteria (Harlow et al., 2012) have been recruited. Exclusion criteria included the following: taking hormone replacement therapy, antidepressants or antianxiety medications, psychotropic drugs, hormone birth control or hormonal supplements. Additional exclusion criteria included being pregnant or nursing, and women with psychiatric disorders including depression, bipolar disorder, psychotic disorder, and alcoholism or drug addiction. The FEMM Study was advertised through multiple online avenues, including on KIJJI, used Regina, and Facebook. Flyers were distributed throughout the University of Regina, in doctor’s offices, gas stations, female gyms, supermarkets, leisure centres, and libraries. Participants were compensated $250 upon completion of the FEMM study.
2.2 Materials

**Structured Clinical Interview for DSM-5 (Research Version; SCID-5-RV; First, Williams, Karg, & Spitzer, 2015).** The SCID-5-RV is a semi-structured interview for clinicians or trained professionals to use to make diagnoses of mental illnesses based on *DSM-V* criteria (American Psychological Association, 2013). The SCID-5-RV can be used for clinical or general populations. In the current study, the SCID-5-RV was used to identify current and past psychiatric disorders during an enrollment session for eligibility purposes. The SCID-5-RV was administered by trained research assistants.

**Center for Epidemiological Studies Depression Scale (CES-D; Radloff, 1977).** The CES-D is a 20-item scale on which participants rated each item based on frequency (i.e., 0 = rarely to 3 = most or all the time). The scale was designed to measure depressive symptoms (Radloff, 1977). The CES-D has good internal consistency, Cronbach’s α ranging from .85-.91 within different ethnic contexts (Radloff, 1977; Roberts, 1980) and test-retest variability with values above .50 for cancer patients (Hann, Winter, & Jacobsen, 1999). This self-report scale was used to measure depressive symptoms as a covariate and was completed online in Qualtrics.

**Sleep diary.** Participants completed a sleep questionnaire in Qualtrics. Information gathered included the time they went to bed and woke up, how long it took to fall asleep, amount of times the participants woke up through the night, how long they stayed up after a nighttime awakening, whether they woke up earlier than normal (and how much earlier), length of sleep, and quality of sleep. Questions were selected from the Pittsburgh Sleep Quality Index (PSQI) (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). The PSQI has high internal consistency for individual items with an overall Cronbach’s α at approximately 0.83 (Buysse et al., 1989). This
questionnaire will give us a detailed overview of a participant’s sleep efficacy and will be examined as a possible covariate.

**Hot flash diary.** The hot flash diary asked participants to report their experiences and number of VMS for 24 hours. Participants rated the severity of each hot flash experienced (i.e., mild, moderate, or severe hot flashes). In a Qualtrics survey, participants reported the total number of each severity of hot flashes they have experienced. Using this information, a VMS score was created using the following formula: 

\[
(\text{# of mild VMS} \times 1) + (\text{# moderate VMS} \times 2) + (\text{# severe VMS} \times 3).
\]

The survey also asked participants to rate on a scale from 0 to 3 how bothered they were by their current hot flashes and night sweats.

**Enzyme immunoassays.** We used Salimetrics cortisol ELISA kits (made in State College Pennsylvania). Assay procedures were that which Salimetrics has outlined. Samples were run in duplicate to make sure we could obtain an average reading for higher precision. If the two well samples were more than 15% different from each other they were rerun to ensure accuracy. Samples were stored in a locked freezer at -20 degrees Celsius until assays were completed.

### 2.3 Procedure

**Enrollment Session.** After prospective participants expressed interest in the study, individuals were contacted and screened via telephone. Eligible participants were asked to come to the University of Regina to complete an enrollment session. If an on-site enrollment session was inconvenient for the participant, it was conducted over the phone. The *SCID-5-RV* for the *DSM-V* (Research Version; *SCID-5-RV*; APA, 2013) was administered to determine if the participant had a current psychiatric diagnosis. During this session participants gave their informed consent upon confirmation of eligibility. After informed consent was obtained, participants were given instructions on the procedure for their saliva samples and given a kit for
their saliva collection for the next 12 weeks. The kit contained a cooler with 24 labeled test tubes, two ice packs, and 12 straws.

**Weekly assessments.** Once a week for 12 weeks, participants provided two saliva samples: one immediately upon awakening and another 30 minutes later. Instructions were given to avoid consuming food or drink other than water between the saliva samples. Once the saliva samples were collected, participants stored them in their home freezer. On the same day of the saliva samples, participants completed a measure of their depressive symptoms (i.e., CES-D) and rated the quality of their sleep from the previous night. A 24-hour hot flash diary was also begun the evening prior to the saliva collection day (at 6 pm). The total number of each type of hot flashes (i.e., mild, moderate, and severe) were reported by participants on the evening of the saliva collection day, following completion of the 24-hour period.

### 2.4 Statistical Analyses

Linear mixed models (PROC MIXED in SAS 4.3) were performed to examine the within-subject effects of past-week VMS change on weekly CAR. The same technique was used to examine the within-subject effect of past-week CAR change on VMS. Weekly depressive symptoms and sleep quality were included as covariates, as were previous-week levels of the outcome variable.

### Results

#### 3.1 Participant Characteristics

Participants included in the study ($N = 13$) were “very” or “extremely” bothered by VMS, the majority being “very” bothered by their VMS. Sleep quality among the majority of participants was moderate (i.e., neither very good or very bad). On average, participants were below the cut-off score for the CES-D, indicating few cases of clinically significant depressive
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symptoms. All severities of VMS (i.e., mild, moderate, and severe) were experienced at least two times a week on average, with moderate VMS occurring most frequently. Baseline and weekly characteristics indicate that as intended, the main issue participants were facing in the menopause transition was VMS. For detailed information refer to Table 1.

3.2 Weekly Change in CAR Predicting VMS

There was a statistically significant interaction between the predictive CAR change and weekly number of severe VMS $\beta$(SEM) = -0.55(0.24), $p = 0.02$ such that a negative correlation was present between CAR and the number of severe VMS week to week (see Figure 1). There was no statistically significant relationship between CAR change and number of mild VMS ($\beta$(SEM) = -0.15(0.25), $p = .55$), moderate VMS ($\beta$(SEM) = 0.24(0.29), $p = .42$), total number of VMS ($\beta$(SEM) = -0.56(0.40), $p = .14$), or VMS score ($\beta$(SEM) = -1.46(0.84), $p = .08$)

3.3 Weekly VMS Change Predicting CAR

Three variants of VMS predicted morning cortisol levels. There was a statistically significant negative relationship between week-to-week change in number of severe VMS predicting CAR $\beta$(SEM) = -0.06(0.01), $p < 0.01$, number of total VMS predicting weekly CAR $\beta$(SEM) = -0.05(0.02), $p < 0.03$, and VMS score on weekly CAR $\beta$(SEM) = -0.06(0.02), $p < 0.01$ (see Figures 2, 3, 4 respectively). However, neither mild VMS change ($\beta$(SEM) = -.01(0.02), $p = .70$), nor moderate VMS change ($\beta$(SEM) = 0.01(0.02), $p = .97$) predicted weekly CAR.

Discussion

4.1 Conclusion

This study aimed to clarify the direction of the relationship between cortisol levels and VMS. In concordance with the hypothesis, weekly change in VMS was a stronger predictor of the CAR than the CAR was for VMS. This was evident as many indicators of week-to-week change in VMS were associated with weekly CAR: the total number of VMS, number of severe
VMS, and VMS score were all predictive of the CAR. In contrast, week-to-week CAR change was only associated with the number of weekly severe VMS and no other indicators of VMS severity.

The current study observed a relationship between VMS and a blunted CAR. This is consistent with the study by Gibson et al. observing a flattened 24-hour cortisol profile but in contrast with Rubin et al., (2014), which found that VMS are associated with higher cortisol levels 15 minutes, 30 minutes, and 45 minutes upon awakening. In regard to the directional relationship, the results favouring VMS predicting cortisol is similar to the study by Meldrum et al. (1984). Meldrum found that cortisol increased immediately after and during a hot flash.

In light of Meldrum’s (1984) study finding that an acute spike in cortisol is triggered by hot flashes, it may be that suffering from frequent VMS leads to chronic activation of the HPA axis; chronic activation of the HPA axis can lead to its dysregulation as receptors at the level of the hypothalamus, pituitary gland and adrenal cortex become desensitized and down-regulate (McEwen, 1998). These processes would then lead to a blunted CAR (Fries et al., 2009). Chronic HPA axis activation may also be indirectly triggered by frequent VMS, which can be very disruptive, uncomfortable, and even anxiety-provoking for some. It is therefore plausible that having severe, frequent VMS triggers chronic HPA activation by increasing psychological stress. Another possibility is that VMS triggers HPA axis dysregulation through disrupted sleep; although we did include sleep quality as a covariate in our analyses, our measure may have been insufficiently detailed to fully capture VMS-related sleep disruption.

A second possible mechanism explaining how VMS would be related to cortisol alterations may be related to estrogen’s effects on the hypothalamus, which may contribute both to HPA axis regulation and temperature regulation. VMS are related to low estrogen
concentrations (Santoro, 2017). It has been hypothesized that the estrogen fluctuations which characterize the menopause transition may have effects on thermoregulation (Deecher & Dorris, 2007). There are three components to temperature regulation; the brain, the peripheral vasculature, and the internal body temperature. Core body temperature (CBT) is maintained through constriction of the vessels for heat dissipation and dilation of the vessels for heat conservation. Rather than VMS causing elevated temperature, Deecher and Dorris suggest that there is an issue in the signals to areas that regulate CBT (e.g., the hypothalamus). This results in over-dilation of the vessels which causes sweating and eventual chills. The anterior hypothalamus, a major structure within the HPA axis, plays an important role in the thermoregulation process. The fluctuations of estrogen can affect hypothalamic functioning, specifically with temperature regulation. It is therefore possible that estrogen’s effect on the hypothalamus could simultaneously influence the HPA axis’ release of diurnal cortisol levels as well as influence core body temperature to increase the likelihood of VMS.

Although the main finding of the current study was that increases in VMS predict HPA axis dysregulation, there was also some evidence that HPA axis dysregulation may be a trigger for increased VMS. This finding is consistent with the suggestion that HPA axis dysregulation may alter the hypothalamus’ regulation of body temperature, thereby increasing one’s susceptibility to experiencing VMS (Deecher & Dorries, 2007).

4.2 Limitations and Future Directions

The current study should be interpreted in light of some limitations. First, as there were only thirteen participants who were significantly bothered by their hot flashes, this may affect the generalizability of our findings. Second, the study is heavily dependent on participant’s adherence to the weekly procedures. However, since most of the surveys are online, we were
able to verify the date and time at which surveys are completed; unfortunately, this was not the case with the saliva samples. It is therefore possible that some saliva samples were not taken at exactly the correct times, which is problematic when attempting to capture rapidly changing morning cortisol concentrations. Third, we are unable to differentiate between the frequency of hot flashes versus night sweats; however, some research suggests that night sweats may be more closely related to cortisol alterations than daytime hot flashes (e.g., Gordon et al., 2016). Fourth, VMS frequency and severity were assessed by self-report, which is not as precise as measuring objective VMS using body temperature. It is possible that VMS frequency was higher, but the individual didn’t realize they were experiencing a hot flash. Despite these limitations, the current study has many notable strengths. For example, it is the first of its kind to examine the direction of the relationship between VMS and cortisol using longitudinal data. It is also noteworthy that weekly sleep quality and depressive symptoms were included as covariates to prevent the results being affected by these confounding variables. The repeated measures design is a strength as well.

Future research should examine the mechanistic properties of the VMS-CAR relationship. Studies which examine whether overactivation of the HPA axis is a result of consequence of VMS will provide a better understanding to aid in VMS treatment options. Psychosocial and physiological baseline predictors of the effects of VMS should also be examined. Variables such as trait mindfulness, life stress, existing health conditions, and anxiety could possibly mediate the relationship between VMS and a blunted CAR. In-depth examinations of the baseline characteristics will be able to identify mediator variables between the VMS and CAR interaction.
Additional future research should include whether interventions aimed at treating VMS is successful in restoring a normal diurnal cortisol profile. Furthermore, given the known relationship between HPA axis dysregulation and CVD (Walker, 2007), studies should also examine whether treatments for VMS can reduce the risk of CVD. For example, selective serotonin reuptake inhibitors, hormonal replacement therapy (HRT), and mindfulness-based stress reduction have been shown to be effective VMS treatments that would be of potential interest (Carmody, Crawford, & Churchill, 2006; Carmody et al., 2011; Pinkerton et al., 2017; Stearns, Beebe, Iyengar, & Dube, 2003). Although HRT is currently identified as the most common and effective treatment for VMS, non-pharmacological agents have the advantage of fewer side effects and negative health implications (Pinkerton et al., 2017). Studies should therefore compare pharmacological treatments and behavioural treatments in terms of their effects on a women’s CVD risk profile.
References


Appendix A: Tables

Table 1. Participant Characteristics

Table 1. The mean of baseline measurements at enrollment session and weekly measurements for 12-weeks

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (% or SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline Measures</strong></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>51.38 (2.40)</td>
</tr>
<tr>
<td>Very Bothered by Hot Flashes</td>
<td>56.25%</td>
</tr>
<tr>
<td>Extremely Bothered by Hot Flashes</td>
<td>43.75%</td>
</tr>
<tr>
<td>Very Bothered by Night Sweats</td>
<td>56.25%</td>
</tr>
<tr>
<td>Extremely Bothered by Night Sweats</td>
<td>43.75%</td>
</tr>
<tr>
<td>Very Good Sleep Quality</td>
<td>6.25%</td>
</tr>
<tr>
<td>Fairly Good Sleep Quality</td>
<td>43.75%</td>
</tr>
<tr>
<td>Fairly Bad Sleep Quality</td>
<td>37.50%</td>
</tr>
<tr>
<td>Very Bad Sleep Quality</td>
<td>12.50%</td>
</tr>
<tr>
<td><strong>Weekly Measurements</strong></td>
<td></td>
</tr>
<tr>
<td>CAR (ng/ml)</td>
<td>0.16 (0.18)</td>
</tr>
<tr>
<td>CES-D Total Score</td>
<td>10.12 (8.56)</td>
</tr>
<tr>
<td>Number of Mild VMS</td>
<td>2.80 (1.59)</td>
</tr>
<tr>
<td>Number of Moderate VMS</td>
<td>3.03 (2.41)</td>
</tr>
<tr>
<td>Number of Severe VMS</td>
<td>2.07 (1.71)</td>
</tr>
<tr>
<td>Number of Total VMS</td>
<td>7.90 (4.54)</td>
</tr>
<tr>
<td>VMS Score</td>
<td>15.07 (9.69)</td>
</tr>
</tbody>
</table>
Figure 1. The predicted relationship between week-to-week change in the CAR and weekly number of severe VMS. A negative relationship was observed. CAR was obtained via morning saliva samples and VMS frequency was obtained via self-report for a 24-hour period. $N = 13$. 

Figure 1. Predicted Relationship of Past-Week Change in CAR and Severe VMS
Figure 2. The relationship between the predictive week-to-week change in the number of severe VMS and weekly CAR concentrations. A negative relationship was observed. CAR was obtained via morning saliva samples and VMS frequency was obtained via self-report for a 24-hour period once a week for 12 weeks. \( N = 13 \).
Figure 3. Predicted Relationship of Past-Week Change in Total VMS and the CAR

Figure 3. The relationship between the predictive week-to-week change in the number of total VMS and weekly CAR concentrations. A negative relationship was observed. CAR was obtained via morning saliva samples and VMS frequency was obtained via self-report for a 24-hour period once a week for 12 weeks. \( N = 13 \).
Figure 4. The relationship between the predictive week-to-week change in the VMS score and weekly CAR concentrations. A negative relationship was observed. CAR was obtained via morning saliva samples and VMS frequency was obtained via self-report for a 24-hour period once a week for 12 weeks. N = 13.
Appendix C: CES-D

Below is a list of some ways you may have felt or behaved. Please indicate how often you have felt this way during the past week:  (circle one number on each line)

<table>
<thead>
<tr>
<th>During the past week…</th>
<th>Rarely or none of the time (less than 1 day)</th>
<th>Some or a little of the time (1-2 days)</th>
<th>Occasionally or a moderate amount of time (3-4 days)</th>
<th>All of the time (5-7 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I was bothered by things that usually don’t bother me……………</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2. I did not feel like eating; my appetite was poor……………………</td>
<td>0</td>
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</tr>
<tr>
<td>3. I felt that I could not shake off the blues even with help from my family ………………………</td>
<td>0</td>
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</tr>
<tr>
<td>4. I felt that I was just as good as other people……………………</td>
<td>0</td>
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</tr>
<tr>
<td>5. I had trouble keeping my mind on what I was doing……………</td>
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<tr>
<td>6. I felt depressed………………</td>
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<tr>
<td>7. I felt that everything I did was an effort……………………</td>
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<tr>
<td>8. I felt hopeful about the future..</td>
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<tr>
<td>9. I thought my life had been a failure………………………………</td>
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<tr>
<td>10. I felt fearful………………</td>
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<td>11. My sleep was restless……</td>
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<tr>
<td>12. I was happy…………………..</td>
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<td>3</td>
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<tr>
<td>13. I talked less than usual……</td>
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<td>14. I felt lonely……………….</td>
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<tr>
<td>15. People were unfriendly…..</td>
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<tr>
<td>16. I enjoyed life……………….</td>
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<tr>
<td>17. I had crying spells…………….</td>
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<td>3</td>
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<tr>
<td>18. I felt sad…………………….</td>
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<tr>
<td>19. I felt that people disliked me..</td>
<td>0</td>
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</tr>
<tr>
<td>20. I could not get “going”………</td>
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</table>