Symptoms during the Menopausal Transition and Early Postmenopause and their Relation to Endocrine Levels over Time: Observations from the Seattle Midlife Women's Health Study

Nancy Fugate Woods, RN, PhD, FAAN[1]

Kathleen Smith-DiJulio, RN, MA, PhC[1]

Donald B. Percival, PhD[2]

Eunice Y. Tao, MD (foreign)[1]

Heather J. Taylor, BS[3]

Ellen Sullivan Mitchell, RN, PhD[1]

From: [1]Department of Family and Child Nursing, [2] Applied Physics Laboratory, [3] Department of Biobehavioral Nursing and Health Systems, University of Washington, Seattle, WA, 98195

Corresponding author:

Nancy Fugate Woods, RN, PhD, FAAN

Department of Family and Child Nursing, University of Washington School of Nursing

T318, Health Sciences Bldg., Box 357260

Seattle, WA 98195-7260

(206) 221-2472; FAX: (206) 543-4091

E-mail: nfwoods@u.washington.edu

Running title: Menopausal transition symptoms and hormones

Abstract

Objective: Determine whether hot flashes, depressed mood, sleep, cognitive and sexual symptoms correlate with urinary follicle-stimulating hormone (FSH), estrone (E_1G) and testosterone (T) and with each other during the menopausal transition and early postmenopause (PM).

Design: Forty-one women who transitioned from middle or late transition stage to PM, rated symptoms and provided monthly urine specimens as part of a longitudinal study of the menopausal transition were included in the analyses.

Results: Correlations between endocrine levels and symptom severity ratings over time revealed that hot flash severity was significantly and positively related to FSH and negatively to E_1G. Vaginal dryness was positively correlated with FSH and negatively correlated with T. Decreased sexual desire was correlated negatively with E_1G levels. Forgetfulness was positively correlated with FSH; difficulty concentrating was negatively correlated with T. Severity of sleep symptoms and depressed mood were not correlated with E_1G, FSH or T. Correlations among the symptoms revealed that severity of hot flashes was associated with sleep disruption and forgetfulness. Depressed mood was correlated with sleep disruption, difficulty concentrating and decreased sexual desire, but not with hot flashes or vaginal dryness. Awakening during the night was correlated with decreased sexual desire and vaginal dryness, as well as hot flashes. Forgetfulness was associated with hot flashes and difficulty concentrating, whereas difficulty concentrating was associated with depressed mood and early awakening.

Conclusion: Symptoms many women experience during the menopausal transition and early PM are uniquely related to different endocrine levels (FSH, E_1G, and T). Sleep disruption

may be key to understanding the relationships among symptoms, menopausal transition and early PM.

Key words: menopause, menopausal symptoms, postmenopausal women, sleep, hot flashes

Introduction

The relationship among reproductive endocrine levels and severity of symptoms women experience during the transition to menopause and early postmenopause (PM) has captured the interest of clinicians, investigators and women themselves. Women attribute many types of symptoms to the menopausal transition: hot flashes, sweats, sleep disruption, mood changes and changes in sexual desire[1]. In the post Women's Health Initiative era, clinicians and researchers search for new symptom management approaches for women with severe symptoms[2,3], and women, themselves, demonstrate increasing interest in complementary and alternative medicine as therapies[4].

Hot flashes and sweats increase in prevalence as women approach the final menstrual period[5]. In longitudinal studies of midlife women, vasomotor symptoms were associated with leutenizing hormone (LH) pulses[6,7], low estradiol[8, 9,10], low inhibin levels[8], and high levels of follicle-stimulating hormone (FSH)[8-11]. Both serum-hormone binding globulin (SHBG) and free estradiol levels (FEI) were also associated with a lower prevalence of hot flashes[10]. In one study increased androgen levels were associated with a decreased frequency of postmenopausal vasomotor symptoms[12]. In acute studies of hot flashes where laboratory stimuli were used to induce the symptom, elevations in LH, adrenocorticotropic hormone (ACTH), gonadotropic hormone (GH), and cortisol all were closely associated in time with the experience of the hot flash, but no changes were reported in estradiol and FSH levels[13,14].

During the menopausal transition women also report perceptions of disrupted sleep. They commonly attribute these disruptions to hormonal changes, and hot flashes, which they believe cause awakening[1]. In contrast to women's explanatory models for sleep symptoms, evidence from recent studies showed that women awaken, then have a hot flash[15]. Sleep symptoms have been associated with increased FSH during the late reproductive stage and to increased levels of pregnanediol and FSH in the perimenopause[16]. In another study, lower

estradiol levels were associated with sleep symptoms among women who were still cycling regularly[17].

Recently reported longitudinal study results indicate an association of higher FSH and LH levels and increased variability of estradiol, FSH, and LH (within women) with depressed mood symptoms[18,19], as well as lower levels of estradiol[19,20] and dehydroepiandrosterone (DHEAS)[21,22]. Whether an increase in the incidence of depressed mood occurs as women traverse the menopausal transition and whether the early or late menopausal transition stages are times of vulnerability to depression remain to be determined[19,23-25].

Although women often notice cognitive symptoms, which they describe as forgetfulness or difficulty concentrating, few women rate these symptoms as serious[26]. They do attribute these symptoms to changing hormones as well as general aging and life stress[27]. To date cognitive symptoms have not been studied with a longitudinal design, although the Study of Women Across the Nation (SWAN) has reported stage-specific prevalence of forgetfulness ranging from 31 percent during the late reproductive stage to 42 percent in the PM[28]. The single longitudinal assessment of cognitive functions indicated that the changes were related to aging[29]. Nonetheless, there has been widespread interest in the use of hormone therapy to prevent cognitive decline and little evidence of efficacy[30,31]

To date there are a few reports from longitudinal studies of the relationship between hormone levels and changes in sexual desire[32,34]. Low estradiol levels have been associated with dyspareunia[34], and lower sexual functioning scores in the Melbourne Midlife Women's Health Study were associated with higher FSH levels and lower estradiol levels[33]. Although there was no relationship with testosterone in the Melbourne study, participants in the Penn Ovarian Aging study who experienced fluctuation in testosterone levels experienced more sexual functioning symptoms[35].

The results of previous work suggest that the hypothalamic-pituitary-ovarian axis hormonal changes occurring during the menopausal transition may be linked to several groups of symptoms, including hot flashes, sleep disturbances, depressed mood, and decreased sexual functioning[36]. Many reports of the relationships between symptoms and endocrine levels were based on cross-sectional analyses of data from women in different phases of the menopausal transition. Moreover, in many of the large longitudinal studies, the estimates of endocrine levels are based on an annual blood draw in which serum levels of hormones with a pulsatile release pattern form the bases for the estimate. The Seattle Midlife Women's Health Study (SMWHS) has focused on the transition to menopause for a smaller number of women than the large studies but has collected urinary hormone levels based on a first morning sample several times each year of the study, up to nine years[37]. The ability to study these phenomena with frequent, repeated measures over several years as a part of a prospective, longitudinal study of the menopausal transition provides the opportunity to conduct intensive within woman analyses of the relationships among symptoms and endocrine change over the menopausal transition.

The purposes of this study were to:

1) Determine the relationship between urinary endocrine levels (E_1G, FSH, and T) and the severity of symptoms (hot flashes, sleep disruption, depressed mood, sexual and cognitive) women experience during the menopausal transition.

2) Determine the relationships among symptoms experienced during the menopausal transition, specifically, hot flashes, early awakening, waking during the night, depressed mood, forgetfulness, difficulty concentrating, decreased sexual desire and vaginal dryness.

Methods

Design and Sample. The data for these analyses are part of the SMWHS, a larger longitudinal study. Women (n=508) were recruited between 1990 and 1992 when most had

not yet begun the menopausal transition or were in the early stages of the transition to menopause[38]. After completing an initial in-person interview administered by a trained registered nurse interviewer, 367 women agreed to provide data annually by questionnaire, daily menstrual calendar, and health diary. At the end of 5 years, 242 women agreed to continue to participate for an additional 5 years and 170 to provide a first morning urine specimen 8 to 12 times per year on day 6 of the menstrual period. Urine specimens were collected monthly from late 1996 through 2000 and quarterly from 2001 through 2005. Forty-one women with a documented final menstrual period were selected for the analyses presented here because they provided at least 10 pairs of symptom and hormone data. Of these women, 24 had a documented entry into the middle and 37 into the late menopausal transition stage. Four women transitioned directly from middle stage to their final menstrual period.

Measures

Menopausal Transition Stages (MTS). Women were classified according to MTS based on daily menstrual calendar data using the method of Mitchell, Woods and Mariella[38], in which menopausal transition was divided into three stages: early, middle, and late transition. In brief, early transition was defined by changes in either menstrual flow or cycle length at age ≥35 years, without changes in cycle regularity. The middle transition was defined by irregularity of more than 6 days absolute difference between any two consecutive menstrual cycles during the reference year, without skipping periods. Late transition was defined by skipping one or more menstrual periods. A skipped period was defined as double the modal cycle length or more for the reference year. In the absence of a modal cycle length, a population-based cycle length of 29 days was used[39]. PM was defined by at least 12 months

without any bleeding or spotting for women who were not taking estrogen replacement or birth control pills.

Urinary Assays. Urinary assays were performed in our laboratories using a first-voided morning urine specimen provided on day 6 of the menstrual cycle, if menstrual periods were identifiable. For women with no bleeding or spotting or extremely erratic flow, a consistent date was used. Women abstained from smoking, caffeine use, and exercise before the urine collection. Urine samples were preserved with sodium ethylenediaminetetraacectic acid and sodium metabisulfite and frozen at –70°C.

All specimens, standards and controls were tested in duplicate and those with a coefficient of variance above 15% were repeated. A BioRad Quantitative Urine control and a pooled in-house urine control were included in all assays, and a member of the standard curve was repeated after every ten unknowns to monitor assay performance. Multiple samples from each subject were included in the same assays during each year. In general, all samples from a calendar year were assayed during the next calendar year.

Urinary creatinine. All endocrine concentrations were corrected for variations in urine concentration by expressing the hormone level as a ratio to the concentration in the same urine specimen. Urine specimens were assayed for creatinine using the method of Jaffe[46]. The inter-assay variation (run to run) was 6.7% and the intra-assay variation was 3.1% (n = 405).

Urinary estrone glucuronide (E_1G). E_1G was used for measuring estrogen because it is stable, can be reliably measured without special preparation, and is highly correlated with serum estradiol levels[40-44]. Urinary E_1G was measured by a competitive enzyme immunoassay (EIA) that cross-reacts 100% with E_1G, 83% with estradiol glucuronide and less than 10% with free estrone, estrone sulfate, estriol glucuronide, estradiol and estriol[42]. Urinary E_1G concentrations estimated from this EIA were highly correlated with serum

estradiol (Pearson's r=0.95; based on 30 averaged cycles)[42]. The lower limit of detection for the assay was 3.1 nmol/L. Average recovery from a urine matrix of low, medium and high E_1G standard doses was 101% [42]. Intra- and inter-assay coefficients of variation (CV) were 2.1% and 9.6%, respectively, for an external (BioRad) urine control (mean concentration 2.1ng/mL); and 2.8% and 14.5%, respectively, for an internal urine control (mean concentration 1.59 ng/mL) (determined using the method of Rodbard[45] from 20 randomly selected plates). There was no evidence of trending in the urine control specimens across the study: the inter-assay CV from 255 microtiter plates was 10.1% for the internal control and 10.8% for the external control. E_1G concentration was estimated from absorbance (Dynatech MR7000 Plate reader, test wavelength 405 nm; reference wavelength 570 nm) using a four parameter logistic in Biolinx 1.0 Software (Dynex Laboratories). E_1G concentrations were corrected for slight assay non-parallelism[42] and standardized to a 1:5 dilution for all specimens.

Urinary FSH. FSH was assayed using Diagnostic Products Corporation (DPC) Double Antibody FSH Kit. This radioimmunoassay (RIA) was designed for the quantitative measurement of FSH in serum and urine. An extraction of urinary FSH was done to achieve the necessary sample concentration and to insure matrix compatibility between the calibrator and the unknowns. Recoveries for extraction were in the order of 95%. The DPC protocol for extracting the FSH from the urine specimen used sodium acetate and spectrophotometric grade acetone. Following an overnight freeze, the FSH was precipitated by centrifugation and the solvent was removed by aspiration in a fume hood. The precipitate was dried under a gentle stream of nitrogen and re-suspended in an FSH-Zero Calibrator. The Zero-Calibrator was bovine serum validated to contain zero levels of FSH by DPC prior to release. The assay was a competitive liquid phase kinetic assay in tube format. Re-suspended extracts were incubated with an anti-FSH antibody, then in a second incubation ^{125}Iodine-labeled FSH

competes for anti-FSH antibodies in solution. The antibody-bound ^{125}Iodine-labeled FSH was precipitated with a second anti-FSH antibody. All unbound ^{125}Iodine FSH remaining in the supernatant was removed by aspiration.

The FSH levels were determined by measuring counts per minute using a Cobra Series Auto-Gamma Counting System. Unknown FSH levels were inversely related to tube counts and interpolated from a logit-log representation derived from calibrators ranging from 2 to 100 mIU/ml. Standards ranging from 5 to 40 mIU/mL were used throughout the study to calculate variance[47]. FSH levels used in the analyses were reported as mIU/mg creatinine. The reporting range for urine FSH was 2.0 to100 mIU/mL, the minimum detectable concentration was 1.6 mIU. The inter-assay variation (run to run) was 7.1% and the intra-assay variation (within run) was 3.7% (N=205).

Urinary testosterone (T). Testosterone levels were assayed using the DPC Total Testosterone Kit, a solid-phase RIA using a testosterone- specific antibody immobilized to the wall of a polypropylene tube[48-50]. A hydrolysis step preceded the assay and was accomplished by addition of concentrated hydrochloric acid to the urine specimens. The acidified samples were then boiled for 15 minutes to remove any interfering substances from the urine. The hydrolyzed urine was diluted into a testosterone-free zero matrix, incubated in the tubes and then evaluated by RIA. Labeled ^{125}I-testosterone was added to all samples and competed with the T present in the sample for binding sites on the tube walls during a three-hour incubation at 37C. The unbound testosterone was decanted and the remaining ^{125}I-testosterone determined by measuring counts per minute using a Cobra Series Auto-Gamma Counting System as with FSH. The calibration curve was prepared using standards ranging from zero to 400ng/dl. The inter-assay variation (run to run) was 12.38% (N=791) and the intra-assay variation (within run) was 8.75%. Standards ranging from 25 to 400ng/dl were used throughout the study to calculate recovery. The average recovery was 92.7% and ranged

from 86.1 to 106%. T levels used in the analysis were reported as ng testosterone per mg creatinine.

Symptoms. Symptoms were measured with the 3-day diary. Women rated each symptom on a scale from 0 (not present) to 4 (extreme) for the past 24 hours. Ratings were averaged over 3 days spanning the date of urine sample collection. Hot flashes, depressed mood, cognitive symptoms of forgetfulness and difficulty concentrating, sleep symptoms of early morning awakening and awakening during the night, and decreased sexual desire and vaginal dryness were analyzed separately.

Analyses

Intra-individual analyses of repeated measures of symptoms and endocrine values were the primary foci of these analyses. To test the null hypothesis that there was no association between a particular symptom and a particular assay (or another symptom) we used a nonparametric procedure consisting of Spearman's rho combined with a sign test (the latter is the same as a binomial test in which both outcomes are regarded as equally likely). For a given woman, symptom and assay data obtained within 3 days of one another were paired. Spearman's rho was computed for the paired data for each woman. This measure of association was used rather than the more familiar Pearson product moment correlation coefficient because both the assay and its log transform had heavy-tailed distributions, which can cause the sample correlation coefficient to be inordinately influenced by a small number of data points. Under the null hypothesis, any deviation from zero in computed rhos was assumed to be equally likely to be positive or negative. The sign (binomial) test was calculated to determine whether the ratio of positive Spearman's rhos to the total number of rhos was significantly greater or less than what was expected by chance, i.e., 1/2. There were no instances in which the computed rho was exactly zero.

Results

Participants: Data were obtained from a total of 41 women who entered the study between 1990 and 1992 and provided menstrual calendar, diary symptom and assay data on at least ten occasions spanning their middle transition stage to at least one year PM. None of the women was taking exogenous estrogens, progestins, selective estrogen receptor modulators (SERMS) or corticosteroids. The 41 women meeting study inclusion criteria compared to those not meeting criteria (n=201) were similar with respect to age, years of education, BMI, income level, employment status, race/ethnicity, and partnered/married status. As seen in Table 1 there were no significant differences between these two groups of women.

Correlations between Hormones and Symptoms: Table 2 shows the relationship between hormone levels and symptom severity and provides the average Spearman's rho and its standard deviation, the ratio of positive rhos to the total number of rhos, and the associated confidence intervals and p value for the sign test for all women with data for these analyses. The observed rhos between FSH and hot flash severity averaged 0.24 and were positive in 32 of 40 women. The sign test indicated that the population measure of association (rho) between FSH and hot flash severity was significantly different from zero ($p < 0.001$). The observed rhos between E_1G and hot flash severity were negative in 36 of 40 women with an average of -0.29 and a significant sign test ($p < 0.001$). T was not significantly cross-correlated with hot flash severity. Rhos between E_1G and severity of early morning awakening and waking during the night, as well as testosterone and severity of both symptoms of sleep disruption trended in a negative direction but were not significant. Rhos between FSH and severity of symptoms of sleep disruption were slightly positive on average, but not greater than would have occurred by chance.

The number of positive rhos between severity of depressed mood and E_1G, FSH and T was no greater than expected by chance (22 of 39, 23 of 40, and 19 of 40 respectively). The rhos between E_1G and decreased sexual desire were negative in 24 of 35 women with a significant

sign test ($p = 0.04$) even though the average rho (-0.07) was weak (s.d. = 0.20). Neither T nor FSH was significantly related to decreased sexual desire.

The rhos between testosterone and vaginal dryness were negative in 21 of 30 women, leading to a significant sign test ($p = 0.04$) but with a weak average rho of -0.03 (s.d. = 0.18). Conversely, the rhos between FSH and vaginal dryness were positive in 23 of 30 women ($p = 0.005$), with a mean value of 0.114 (s.d. = 0.221).

The rhos between T and difficulty concentrating were negative in 25 of 36 women (mean rho = -0.05, s.d. = 0.16, $p = 0.03$). The rhos between FSH and forgetfulness were positive in 23 of 33 women (mean = 0.06, s.d. = 0.14, $p = 0.04$). E_1G was not significantly related to forgetfulness or difficulty concentrating.

Correlations between Symptoms: In addition to examining the associations between endocrine values and symptom severity ratings, the relationship between the symptoms themselves was of interest. Table 3 displays the sample mean values and standard deviations for each woman's Spearman's rho. As with Table 2, the ratio of positive to total number of rhos, confidence intervals and p values are given.

Hot flashes and both symptoms of sleep disruption had positive rhos in 26 of 38 women for early awakening (mean = 0.08, s.d. = 0.24, $p = 0.03$) and 31 of 38 women for waking during the night (mean = 0.20, s.d. = 0.24, $p < 0.001$). Hot flashes were also positively associated with forgetfulness in 23 of 33 women (mean rho = 0.10, s.d. = 0.18, $p = 0.04$). The number of positive rhos between hot flashes and difficulty concentrating, as well as hot flashes and depressed mood, decreased sexual desire and vaginal dryness was no greater than would have occurred by chance.

Both symptoms of sleep disruption were significantly associated with each other (in 33 of 37 women, mean rho = 0.34, S.D. = 0.24, $p < 0.001$) and with severity of depressed mood - early morning awakening in 32 of 41 women (mean rho = 0.19, s.d. = 0.21, $p < 0.001$) and waking

during the night in 25 of 37 women (mean rho = 0.09, s.d. = 0.18, p = 0.05). There were also positive associations between waking during the night and decreased sexual desire in 22 of 32 women (mean rho = 0.11, s.d. = 0.23, p = 0.05) and vaginal dryness in 20 of 27 women (mean rho = 0.17, s.d. = 0.29, p = 0.02). Early awakening was related to difficulty concentrating in 24 of 34 women (mean rho = 0.08, s.d. = 0.18, p = 0.24).

In addition to being associated with sleep difficulties, depressed mood was positively related to decreased sexual desire (26 positive rhos in a sample of 35 women, mean = 0.23, s.d. = 0.26, $p < 0.001$) and difficulty concentrating (31 of 35 women, mean rho = 0.28, s.d. = 0.23, $p < 0.001$).

Not surprisingly, decreased sexual desire was consistently positively associated with vaginal dryness in 21 of 28 women (mean rho= 0.14, s.d. = 0.21, p = 0.01) and forgetfulness with difficulty concentrating in 30 of 32 women (mean rho=0.31, s.d. = 0.17, $p < 0.001$).

In summary, Spearman's rhos between endocrine levels and symptom severity ratings for women spanning the middle stage of the menopausal transition to PM revealed that:

- Severity of hot flashes was positively associated with FSH and negatively with E_1G.
- Vaginal dryness was positively associated with FSH; negatively associated with T.
- Severity of early morning awakening, awakening during the night and depressed mood were not associated with E_1G, FSH or T.
- Decreased sexual desire was associated negatively with E_1G levels.
- Forgetfulness was positively associated with FSH; difficulty concentrating was negatively associated with T.

In addition, rhos among the symptoms revealed that all trended in the positive direction:

- Severity of hot flashes was associated with early morning awakening, awakening during the night, and forgetfulness.

- Early awakening and awakening during the night were associated with depressed mood, as well as hot flashes; awakening during the night was also associated with decreased sexual desire and vaginal dryness.
- Depressed mood was associated with both symptoms of sleep disruption as well as difficulty concentrating and decreased sexual desire, but not hot flashes or vaginal dryness.
- Forgetfulness was associated with hot flashes and difficulty concentrating, whereas difficulty concentrating was associated with depressed mood and early awakening.
- Decreased sexual desire was associated with awakening during the night, depressed mood and vaginal dryness.
- Vaginal dryness was associated with decreased sexual desire and awakening during the night.

Discussion

Taken together, these findings suggest that the symptoms many women experience during the menopausal transition are uniquely related to different endocrine levels (FSH, E_1G, and T). Indeed, in the women studied here, early morning awakening, awakening in the night, and depressed mood were not related significantly to any of the endocrine values studied. In addition, hot flashes were related to only the sleep symptoms and forgetfulness, but not to depressed mood as has been suggested in other studies[18,19].

The relationship of FSH to vasomotor symptoms has been seen in studies in addition to the SMWHS. Most recently the SWAN study revealed findings of a positive relationship between FSH and hot flashes. Hypothesized mechanisms for vasomotor symptoms include neuroendocrine (alpha adrenergic and central noradrenergic) mechanisms[6,51]. In addition, E_1G was associated with hot flashes in the SMWHS cohort, similar to findings in the SWAN cohort. Randolph and associates found that estradiol had less effect than FSH on hot flashes,

suggesting that nonsteroidal feedback systems are more relevant to the severity of hot flashes than estradiol and that neuroendocrine/gonadal peptide therapies for hot flashes may warrant future attention of investigators[52]. In the Seattle study, severity of hot flashes was not significantly related to T, although in a European cohort serum testosterone was related to a decrease in hot flashes among women during the PM[12,53].

We did not find that women with higher levels of urinary E_1G had less severe sleep disruption, inconsistent with results of Hollander's report from the Penn Ovarian Aging Study which revealed that women who were still having regular cycles but who had lower estradiol levels were more likely to have sleep disruption[17]. In contrast, Kravitz found sleep problems associated with higher FSH levels in women during the late reproductive stage and with higher pregnanediol and higher FSH levels in women during perimenopause[16].

The lack of a relationship between severity of depressed mood and levels of endocrine hormones is unique in our data. Elevated FSH and lower estradiol levels were associated with depressed mood as measured by the Center for Epidemiologic Studies, Depression (CESD) Scale and with a clinical interview in the Penn Ovarian Aging Study, but the study is limited by the small number of women in the late transition phase and PM[18,19]. As a result, it is difficult to determine whether these relationships will be sustained among their cohort as more women progress through the menopausal transition to the PM.

As E_1G becomes the predominant estrogen for women traversing the menopausal transition our finding of a consistent negative relationship between E_1G and decreased sexual desire would seem to corroborate Dennerstein et al's [32,33] findings associating decreased estrogens, not androgen, with changes in sexual functioning. While T was not significantly associated with sexual desire in our study, it was negatively associated with vaginal dryness and FSH was positively associated with vaginal dryness. It is possible that T effects on vaginal dryness observed in our study may indicate that androgens, which are metabolic precursors to

estrogen synthesis, may be influential as women traverse the menopausal transition. Testosterone has been reported to both remain stable and to fluctuate throughout the menopausal transition and early PM[36,53], suggesting the relationship between androgens and sexual function may change over the transition to menopause.

The significant associations between FSH and forgetfulness and testosterone and difficulty concentrating are also of interest in studying cognitive symptoms during the menopausal transition. Earlier studies of cognitive symptoms during this period of a woman's life have not reported relationships between endocrine levels and forgetfulness and difficulty concentrating.

The relationships among symptoms during the menopausal transition are complex and only recently have research reports addressed the relationships among them. Avis and colleagues' longitudinal analysis of the Massachusetts Women's Health Study data revealed that depressed mood, measured by the CESD was predicted by estradiol levels, but when the effect of estradiol was adjusted for symptoms, including hot flashes, the relationship was not significant[20]. Both hot flashes and sleep symptoms were associated with the depressed mood in the Massachusetts study, prompting Avis[23] to posit a domino effect existed among symptoms, such that hot flashes set in motion sleep disruption which, in turn, led to depressed mood. Others have found that at many points in time during the menopausal transition, depressed mood and sleep symptoms co-occur. In response to the hypothesis that hot flashes caused women to awaken, Freedman and colleagues[8] studied women in laboratory circumstances and found that women awakened prior to having hot flashes. Thus it is possible that waking from sleep precedes vasomotor symptoms and that vasomotor symptoms do not cause awakening. It is also possible that changing hormone levels affect the thermoregulatory mechanisms, causing a drop in core temperature during sleep.

Awakening in response to the temperature change could activate the CNS with a resultant increase in core body temperature that could be perceived as a hot flash.

We found that hot flashes were related to both early morning awakening and awakening during the night as well as forgetfulness, but hot flashes and depressed mood were not directly related. Instead, sleep symptoms were related to depressed mood, as was forgetfulness, difficulty concentrating, and decreased sexual desire. The relationship between hot flashes and forgetfulness does not seem to be mediated by lack of sleep since neither sleep symptom was correlated with forgetfulness. Perhaps it is a function of distraction, i.e., when women have severe hot flashes their attention gets diverted to managing the hot flashes and not attending to other environmental events.

It is possible that disturbed sleep is key in understanding many of the symptoms women experience during the menopausal transition and early PM, and may mediate mood, vasomotor, and cognitive symptoms. Disrupted sleep has been linked to mood disturbances in prior studies of women during the menopausal transition[54, 55] and in women during midlife without respect to menopause[55]. It is possible that the aging processes, as well as conditions such as sleep-disordered breathing, are associated with increased arousals that result in perceptions of disrupted sleep[56]. In turn, sleep disturbances in this population may exacerbate vasomotor symptoms, cognitive symptoms, and depressed mood. This work should be confirmed in polysomnographic studies of sleep among women during the menopausal transition and early PM and accompanied by endocrine studies that can help resolve the question of whether aging or the menopausal transition orchestrates this group of symptoms. An alternative hypothesis warranting testing is that sleep disruption, depressed mood, decreased sexual desire, and cognitive symptoms such as difficulty concentrating may represent indicators of an underlying clinical depression, which we could not assess in this study. An inspection of the CESD scores for the 41 women included in these analyses

revealed that over the period during which they were followed, 29% of the women experienced CESD scores above 16, commonly used as an indicator of the need for an assessment for clinical depression.

Conclusion: While the number of subjects in this study is small, data repeatedly collected spanning the period from the middle transition stage to one or more years PM provides a beginning understanding of how endocrine levels cross-correlated with symptoms and symptoms with each other over time. Until there is further understanding of the relationship of sleep to several of the symptoms reported here, sleep hygiene measures provide women with a low risk self-care strategy that may enhance their sleep, mood, sexual desire, and cognition, and reduce the bother associated with hot flashes.

References

1. Woods NF, Mitchell ES. Anticipating menopause: Observations from the Seattle Midlife Women's Health Study. *Menopause* 1999; 6:167-73.

2. Writing Group for the Women's Health Initiative Investigators Risks and Benefits of Estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative Randomized Controlled Trial. *JAMA* 2002; 288:321-33.

3. The women's Health Initiative Steering Committee. Effects of Conjugated equine estrogen in postmenopausal women with hysterectomy: The Women's Health Initiative Randomized Controlled Trail. *J Am Med Assoc* 2004; 291:1701-12.

4. Carpenter J, Neal J. Other complementary and alternative Medicine Modalities: acupuncture, magnets, Reflexology, and Homeopathy. *Am J Med* 2005;118(12B): 109S-117S.

5. Woods NF, Mitchell ES. Symptoms during perimenopause: prevalence, severity, trajectory, and significance in women's lives. *Am J Med* 2005; 118(12B):14S-24S.

6. Casper R, Yen S, Wilkes M. Menopausal flushes: A neuroendocrine link with pulsatile luteinizing hormone secretion. *Science* 1979; 205:823-5.

7. Freedman R. Hot Flashes: Behavioral treatments, mechanisms, and relation to sleep. *Am J Med* 2005; 118 (12B):124S-130S.

8. Guthrie J, Dennerstein L, Hopper J, and Burger H. Hot flushes, menstrual status, and hormone levels in a population-based sample of midlife women. *Obstet Gynecol* 1996; 88:437-42.

9. Guthrie J, Dennerstein L, Taffe J, Lehert P, Burger H. Hot flushes during the menopause transition: a longitudinal study in Australian-born women. *Menopause* 2005; 12:460-467.

10. Randolph J, Sowers M, Bondarenko I, et al. The relationship of longitudinal change in reproductive hormones and vasomotor symptoms during the menopausal transition. *J Clin Endocrinol Metab* 2005; 90:6106-12.

11. Freeman E, Sammel M, Grisso J, Battistini M, Garcia-Espagna B, Hollander L. Hot flashes in the late reproductive years: risk factors for African American and Caucasian women. *J Women's Hlth & Gender Based Med* 2001; 10:67-76.

12. Overlie I, Moen M, Holte A, Finset A. Androgens and estrogens in relation to hot flushes during the menopausal transition. *Maturitas* 2002; 41:69-77.

13. Meldrum D, Tataryn I, Frumar A., Erlik Y, Lu K, and Judd H. Gonadotropins, estrogens, and adrenal steroids during the menopausal hot flash. *J Clin Endocrinol Metab* 1980; 50:685-9.

14. Tataryn I, Meldrum D, Lu K, Frumar A., Judd H. LH, FSH, and skin temperature during menopausal hot flash. *J Clin Endocrinol Metab* 1979; 49:152-4.

15. Freedman R, Roehrs T. Lack of sleep disturbance from menopausal hot flashes. *Fertil Steril* 2004; 82:138-44.

16. Kravitz H, Janssen I, Santoro N, et al. Relationship of day-to-day reproductive hormone levels to sleep in midlife women. *Archiv Intern Med* 2005; 165:2370-6.

17. Hollander L, Freeman E., Sammel M, Berlin J, Grisso J, Battistini M. Sleep quality, estradiol levels and behavioral factors in late reproductive age women. *Obstet Gynecol* 2001; 98:391-7.

18. Freeman EW, Sammel MD, Liu L, et al. Hormones and menopausal status as predictors of depression in women in transition to menopause. *Arch Gen Psychiatry* 2004; 61:62-70.

19. Freeman E, Sammel M, Lin H, Nelson D. Associations of hormones and menopausal status with depressed mood in women with no history of depression.

Arch Gen Psychiat. 2006; 63:375-82.

20. Avis NE, Crawford S, Stellato R, et al. Longitudinal study of hormone levels and depression among women transitioning through menopause. *Climacteric* 2001; 4:243-9

21. Morrison M, Have T, Freeman E, et al. DHEAS levels and depressive symptoms in a cohort of African American and Caucasian women in the late reproductive years. *Biological Psychiatry* 2001; 50:705-11.

22. Schmidt P, Murphy J, Haq N, Danaceau M, Simpson St. Clair L. Basal plasma hormone levels in depressed perimenopausal women. *Psychoneuroendocrinology* 2002; 27:907-20

23. Bromberger JT, Assmann SF, Avis NE, et al. Persistent mood symptoms in a multiethnic community cohort of pre- and perimenopausal women. *Am J Epidemiol* 2003 158:347-56.

24. Bromberger J, Harlow S, Avis N, et al. Racial/ethnic differences in the prevalence of depressive symptoms among middle-aged women: the Study of Women's Health Across the Nation (SWAN). *Am J Pub Hlth* 2004; 94:1378-85.

25. Bromberger J, Meyer P, Kravitz H, et al. Psychologic distress and natural menopause: a multiethnic community study. *Am J Pub Hlth* 2001; 92:1435-42.

26. Woods NF, Mitchell ES, Adams C. Memory functioning among midlife women: observations from the Seattle Midlife Women's Health Study. *Menopause*. 2000; 7:257-65.

27. Mitchell ES, Woods NF. Midlife women's attributions about perceived memory changes: Observations from the Seattle Midlife Women's Health Study. *J Women's Health Gend Based Med.* 2001; 10:351-62.

28. Gold EB, Sternfeld B, Kelsey JL, et. al. Relation of demographic and lifestyle factors to symptoms in a multi-racial/ethnic population of women 40-55 years of age. *Am J Epidemiol.* 2000; 152:463-73.

29. Meyer PM, Powell LH, Wilson RS, et. al. A population-based longitudinal study of cognitive functioning in the menopausal transition. *Neurology.* 2003; 61:801-6.

30. Shumaker SA, Legault C, Thal L, et al. for the Women's Health Initiative Memory Study Investigators. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. *JAMA* 2003; 289:2651-62.

31. Shumaker SA, Legault C, Kuller L, et al. for the Women's Health Initiative Memory Study Investigators. Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: Women's Health Initiative Memory Study. *JAMA* 2004; 291:2947-58.

32. Dennerstein L, Lehert P. Modeling mid-aged women's sexual functioning: a prospective, population-based study. *J Sex Marital Ther* 2004; 30:173-83.

33. Dennerstein L, Randolph J, Taffe J, Dudley E, Burger H. Hormones, mood, sexuality, and the menopausal transition. *Fertil Steril* 2002; 779(suppl 4):S42-S48.

34. Avis NE, Stellato R, Crawford S, Johannes C, Longcope C. Is there an association between menopause status and sexual functioning? *Menopause.* 2000; 7:297-309.

35. Gracia C, Sammel M, Freeman E, et al. Predictors of decreased libido in women during the late reproductive years. *Menopause.* 2004; 11:144-50.

36. Guthrie JR, Dennerstein L, Taffe JR, Lehert P and Burger HG. The menopausal transition: a 9-year prospective population-based study. The Melbourne Women's Midlife Health Project. *Climacteric* 2004; 7:375-389.

37. Woods N, Carr MC, Tao EY, Taylor HJ, Mitchell ES. Increased urinary cortisol levels during the menopause transition. *Menopause* 2006; 13:212-21.

38. Mitchell ES, Woods NF, and Mariella A. Three stages of menopausal transition from the Seattle Midlife Women's Health Study: toward a more precise definition. *Menopause* 2000; 7:334-49.

39. Chiazze L, Brayer FT, Macisco J, Parker M, and Duffy B. The length and variability of the human menstrual cycle. *JAMA* 1968; 203:377-80.

40. Denari JH, Farinati Z, Casas PRF, Oliva A. Determination of ovarian function using first morning urine steroid assays. *Obstet Gynecol* 1981;58:5-9

41. Stanczyk FZ, Miyakawa I, Goebelsmann U. Direct radioimmunoassay of urinary estrogen and pregnanediol glucuronides during the menstrual cycle. *Am J Obstet Gynecol* 1980;137:443-50

42. O'Connor KA, Brindle E, Shofer JB, Miller RC, Klein NA, Soules MR, et al. Statistical correction for non-parallelism in a urinary enzyme immunoassay. *J Immunoassay Immunochem* 2004;25:259-78

43. Lasley BL, Shidleler SE. Methods for evaluating reproductive health of women. *Occup Med* 1994;9:423-33

44. Wilcox AJ, Baird DD, Weinberg CR, Armstrong EG, Musey PI, Wehman RE, et al. The use of biochemical assays in epidemiologic studies of reproduction. *Environ Health Perspect* 1987;75:29-35

45. Robard D. Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. *Clin Chem* 1974; 20:1255-70.

46. Taussky HH. A microcolorimetric determination of creatinine in urine by the Jaffe reaction. *J Biol Chem* 1954;208:853-61

47. Munro CJ, Stabenfeldt GH, Cragun JR, Addiego LA, Overstreet JW, Lasley BL. Relationship of serum estradiol and progesterone concentrations to the excretion profiles of their major urinary metabolites as measured by enzyme immunoassay and radioimmunoassay. *Clin Chem* 1991;37:838-44

48. Tijssen, P. *Practice and Theory of Enzyme Immunoassays.* Amsterdam: Elsevier Publications, 1985.

49. Chad, T. *An Introduction to RIA and Related Technologies,* 4th Ed. Amsterdam: Elsevier Publications, 1990.

50. Dufau ML, Winters CA, Hattori M, et al. Hormonal regulation of androgen production by the leydig cell. *J. Steroid Biochem*. 1984; 20:161-73.

51. Tataryn I, Lomax P, Bajorek J, Chesarek W, Meldrum D., Judd H. Postmenopausal hot flushes: a disorder to thermoregulation. *Maturitas* 1980; 2:101-7.

52. Randolph J, Sowers M., Bondarenko I, et al. The relationship of longitudinal change in reproductive hormones and vasomotor symptoms during the menopausal transition. *J Clin Endocrinol Metab*. 2005; 90:6106-12.

53. Overlie I., Moen MH, Morkrid L, Skjaeraasen JS, Holte A. The endocrine transition around menopause—a five year prospective study with profiles of gonadotropins, estrogens, androgens and SHBG among healthy women. *Acta Obstet Gynecol Scand* 1999; 78:642-7.

54. Shaver J, Giblin E, Lentz M, and Lee K. Sleep patterns and stability in perimenopausal women. *Sleep* 1988; 11:556-61.

55. Shaver J, and Paulsen V. Sleep, psychological distress, and somatic symptoms in perimenopausal women. *Fam Pract Res J* 1993; 13:373-84.

56. Young, T Rabago, D., Zgierska, A, Austin,D, Laurel F. Objective and subjective sleep quality in premenopausal, perimenopausal, and postmenopausal women in the Wisconsin Sleep Cohort Study. Sleep 2003:26(6):667-72.

Reprint Address

Nancy Fugate Woods, RN, PhD, FAAN

Department of Family and Child Nursing, University of Washington School of Nursing

T318, Health Sciences Bldg., Box 357260

Seattle, WA 98195-7260

(206) 221-2472; FAX: (206) 543-4091

E-mail: nfwoods@u.washington.edu

Legend

Table 1. Comparison of participants included in analyses vs. those not included in analyses (N=242)

Table 2. Relationships between endocrine hormone levels and symptom severity: Spearman's rho, mean (SD), ratio of positive: total number of rhos, 95% confidence interval (CI), p value for sign test

Table 3. Relationships between symptoms: Spearman's rho, mean (SD), ratio of positive: total number of rhos, 95% confidence interval (CI), p value for sign test

Table 1. Comparison of participants included in analyses (meeting criteria for symptom ratings and endocrine data) vs. those not included in analyses (N=243)

	Participants meeting inclusion criteria (n=41)	Participants not meeting inclusion criteria (n=201)	Test Results
Characteristic	**Mean (SD)**	**Mean (SD)**	**T-test**
Age in 1997, Phase II	46.78 (3.37)	46.71 (4.53)	NS
Years of education	16.56 (2.59)	15.71 (2.49)	NS
BMI	24.61 (5.05)	28.07 (7.00)	NS
Income Level	43-45K	41 – 43K	NS
Employment status 1997	**N (%)**	**N (%)**	**Chi Square Test**
Employed	38 (92.7)	165 (87.3)	NS
Not employed	3 (7.3)	24 (12.7)	
Race/ethnicity			
White	38 (92.7)	165 (81.7)	NS
Non-white	3 (7.3)	37 (18.3)	
Marital Status in 1997			
Partnered	22 (53.7)	135 (66.8)	NS
Not Partnered (single, divorced, widowed, separated)	19 (46.3)	67 (33.2)	

Table 2. Relationships between endocrine hormone levels and symptom severity: Spearman's rho, mean (SD), ratio of positive: total number of rhos, 95% confidence interval (CI), p value for sign test

Symptoms	E1G	FSH	Testosterone
Hot flashes			
Rho M (SD)	-0.29 (0.21)	0.24 (0.29)	-0.00 (0.24)
Sign Test:			
+/Total	4/40	32/40	21/40
CI	0.03-0.24	0.64-0.91	0.36-0.68
p value	< 0.001	< 0.001	NS
Early Awakening			
Rho M (SD)	-0.05 (0.24)	0.02 (0.19)	-0.03 (0.20)
Sign Test:			
+/Total	17/38	22/38	15/38
CI	0.28-0.62	0.41-0.74	0.24-0.57
p value	NS	NS	NS
Wake During Night			
Rho M (SD)	-0.07 (0.18)	0.09 (0.19)	-0.02 (0.23)
Sign Test			
+/Total	14/39	23/39	19/39
CI	0.21-0.53	0.42-0.74	0.32-0.65
P value	NS	NS	NS
Depressed Mood			
Rho M (SD)	0.03 (0.22)	0.03 (0.22)	0.17 (0.18)

Sign Test:			
+/Total	22/39	23/40	19/40
CI	0.40-0.72	0.41-0.73	0.32-0.64
p value	NS	NS	NS
↓ Sexual Desire			
Rho M (SD)	-0.07 (0.20)	0.05 (0.25)	-0.03 (0.22)
Sign Test:			
+/Total	11/35	23/36	17/35
CI	0.17-0.49	0.46-0.79	0.32-0.66
p value	0.04	NS	NS
Vaginal Dryness			
Rho M (SD)	-0.06 (0.21)	0.11 (0.22)	-0.03 (0.18)
Sign Test:			
+/Total	11/29	23/30	9/30
CI	0.21-0.58	0.58-0.90	0.15-0.49
p value	NS	0.005	0.04
Forgetfulness			
Rho M (SD)	-0.03 (0.18)	0.06 (0.14)	-0.05 (0.17)
Sign Test:			
+/Total	13/33	23/33	14/33
CI	0.23-0.58	0.51-0.84	0.26-0.61
p value	NS	0.04	NS
Difficulty Concentrating			

Rho M (SD)	-0.01 (0.19)	0.02 (0.22)	-0.05 (0.16)
Sign Test:			
+/Total	16/35	21/36	11/36
CI	0.29-0.63	0.41-0.75	0.16-0.48
p value	NS	NS	0.03

Table 3. Relationships between symptoms: Spearman's rho, mean (SD), ratio of positive: total number of rhos, 95% (CI), p value for sign test

Symptoms	Early Awakening	Wake During the Night	Depressed Mood	↓ Sexual Desire	Vaginal Dryness	Forgetfulness
Hot Flashes						
rho M (SD)	0.08 (0.24)	0.20 (0.24)	-0.02 (0.19)	0.05 (0.22)	0.11 (0.28)	0.10 (0.18)
Sign Test:						
+ /Total	26/38	31/38	17/39	20/34	18/29	23/33
CI	0.51-0.82	0.66-0.92	0.28-0.60	0.41–0.75	0.42-0.79	0.51-0.84
p value	0.03	< 0.001	NS	NS	NS	0.04
Early Awake						
rho (M, SD)		0.34 (0.24)	0.19 (0.21)	0.09 (0.20)	0.05 (0.24)	0.07 (0.21)
Sign Test:						
+ /Total		33/37	32/41	20/33	14/29	13/32
CI		0.75-0.97	0.62-0.89	0.42-0.77	0.29-0.67	0.34-0.74
p value		< 0.001	< 0.001	NS	NS	NS

Wake During Night						
Rho M (SD)			0.09 (0.18)	0.11 (0.23)	0.17 (0.29)	0.03 (0.19)
Sign Test						
+/Total			25/37	22/32	20/27	20/32
CI			0.50-0.82	0.50-0.84	0.54-0.89	0.44-0.79
p value			0.05	0.05	0.02	NS
Depressed Mood				0.23 (0.26)	0.09 (0.25)	0.07 (0.20)
rho M (SD)						
Sign Test:				26/35	19/29	19/33
+ /Total				0.57-0.88	0.46-0.82	0.39-0.74
CI				< 0.001	NS	NS
p value						
↓ Sex Desire						
rho M (SD)					0.14 (0.21)	0.11 (0.23)

Sign Test: +/Total CI p value					 21/28 0.55-0.89 0.01	 19/30 0.44-0.80 NS
Vag Dryness rho M (SD) Sign test: +/Total CI p value						0.09 (0.26) 11/24 0.26-0.67 NS
Forgetfulness rho M (SD) Sign test: +/Total CI p value						