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Cortisol Levels during the Menopausal Transition and Early Postmenopause: Observations from the Seattle Midlife Women's Health Study

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Abstract

Aims—Cortisol levels rise among some women during the late stage of the menopausal transition, but we know little about changes in cortisol levels in relation to menopause-related factors (menopausal transition (MT) stage, urinary estrone glucuronide, testosterone, FSH), stress-related factors (epinephrine, norepinephrine, perceived stress), symptoms (hot flashes, mood, memory and sleep), social factors (income adequacy, role burden, social support, employment, parenting, and history of sexual abuse) and health-related factors (depressed mood, perceived health, physical appraisal, BMI, and smoking). Aims were to examine the influence of menopause-related factors, stress-related factors, symptoms, social, and health-related factors on cortisol levels during the menopausal transition.

Methods—A subset of Seattle Midlife Women's Health Study participants who provided data during the late reproductive, early and late MT stages or early postmenopause (PM) and who were not using hormone therapy or corticosteroids (N=132 women, up to 5218 observations) including menstrual calendars for staging the MT, annual health reports, health diaries, and overnight urine specimens (assayed for cortisol, catecholamines, estrone glucuronide and FSH) between 1990 and 2005 were included. Perceived stress, symptoms, and health behaviors were assessed in a health diary. Health-related and social factors were assessed in an annual health update. Multilevel modeling was used to test effects of menopause- related and other factors on overnight cortisol levels.

Results—When tested with age as a measure of time, menopause-related covariates, including estrone glucuronide (E1G), FSH, and testosterone were associated with significant increases in overnight cortisol levels ($p<.0001$). Likewise, epinephrine and norepinephrine were each associated significantly with overnight cortisol levels ($p<.0001$). In multivariate analyses, E1G, FSH, and testosterone constituted the best set of predictors.

Conclusions—Overnight cortisol levels during the MT were associated with E1G, testosterone, and FSH levels. In addition, they were significantly and positively associated with epinephrine and norepinephrine. MT stage, symptoms, and social, stress-related, and health-related factors had little relationship to overnight cortisol levels when other biological indicators were considered.

Cortisol plays an important role in mobilizing responses to psychobiological load.[1] The diurnal pattern of cortisol is linked to sleep-wake patterns, eating, physical activity, and challenges in life. Beginning in the third decade of life, cortisol levels in women and men increase gradually

with age.[2] Moreover, variability in the circadian pattern of cortisol increases as women age.[2,3]

The integrative functioning of the hypothalamic-pituitary-adrenal (HPA) and the hypothalamic-pituitary-ovarian (HPO) axes is well established, but to date there is limited understanding of the HPA axis functioning during the menopausal transition (MT) and early postmenopause (PM), a time of change in HPO function.[4,5] The early menopausal transition stage is characterized by increasing irregularity in menstrual cycle length, marked by a difference between subsequent cycles of seven or more days. The late stage is marked by skipped periods, with the cycle length exceeding 60 days, which is a cycle length double the modal cycle length or more for the calendar year.[6]

During the late MT FSH rises and women experience an increased incidence and severity of hot flashes.[7] The late MT stage is also a period of midlife during which women experience mood, sleep, and memory symptoms.[8,9] Adrenal activation occurs during the MT, with a transient rise in DHEA-S observed in the SWAN cohort during the late MT stage, but cortisol levels during the MT in the SWAN cohort have not been reported.[10] We recently observed an increase in overnight cortisol levels as women transitioned from the early to the late MT stage.[5]

In addition to the MT, other factors could be associated with a rise in cortisol during the MT. Based on earlier studies, we would hypothesize that cortisol levels would rise with age[2,3], possibly accounting for the increases we observed in in the late MT and reported earlier[7]. In addition, published evidence would support a hypothesis that cortisol levels are elevated in response to both increased endogenous and exogenous estrogen levels[11–15]. Given prior evidence linking cortisol levels to stress exposure, particularly to major life events or prolonged chronic stressors[16–18], we would anticipate a positive association between stressors and cortisol levels. In addition, the past evidence documenting a relationship between major depression and cortisol would prompt us to hypothesize a positive relationship between depressed mood symptoms and cortisol levels[4]. Health practices such as exercise participation, caffeine and alcohol use, and smoking have also been associated to increases in cortisol levels in prior studies[2,3]. Rising cortisol levels have been associated with poor health, including lower bone density in older women[19], minor cognitive complaints[20,21], and could be hypothesized to be related to perceptions of poor health[22].

Early laboratory studies of hot flashes demonstrated a rise in cortisol coinciding with hot flashes measured with sternal skin conductance and temperature[23]. In addition, rising cortisol levels were associated with more severe hot flashes in our earlier report[5].

Given the importance of metabolic syndrome in heart disease, it is important to consider the potential relationship with rising cortisol levels during the menopausal transition. Women in the late menopausal transition stage had higher total cholesterol, greater LDLC, higher apo B levels, and greater VLDL cholesterol levels[24]. Thus changes in cortisol levels across the menopausal transition may be associated with increased risk of metabolic syndrome and potentially associated with later development of diabetes and heart disease.

In order to assess the relationship of menopause-related and other factors to overnight cortisol levels, we sought to:

1. assess whether cortisol levels varied with menopausal transition-related factors, including MT stages, urinary estrone glucuronide, testosterone, and FSH, and hot flash severity; and
2. explore the relationships among menopause-related factors (menopausal transition (MT) stage, urinary estrone glucuronide, testosterone, FSH), in conjunction with

stress-related factors (epinephrine, norepinephrine, perceived stress, history of sexual abuse, role burden, parenting, employment), symptoms (hot flashes, mood, memory and sleep), social factors that may buffer stress (income adequacy, social support) and health-related factors (depression, perceived health, physical appraisal, BMI, and smoking) and cortisol levels during the MT and early postmenopause.

Methods

Design

The data for these analyses are part of a larger longitudinal study, the Seattle Midlife Women's Health Study. Women entered the cohort between 1990 and early 1992 when most were not yet in the MT or were in the early stages of the transition to menopause. After completing an initial in-person interview administered by a trained registered nurse interviewer, participants provided data annually by questionnaire, menstrual calendar, and health diary. In late 1996 a subset of the original cohort began providing 3-day monthly diary data as well as first morning voided urine specimens 8 to 12 times per year for endocrine assays (through 2000), and then quarterly for 2001–2005).

Sample

Participants (N=132) were those who contributed at least one overnight urine sample to be assayed for cortisol starting in 1996. Women whose data were available for analysis were midlife women with a mean age of 40.4 (SD=3.9) years at the beginning of the study, 16.0 years of education (SD=2.8), and a median family income of $41,400 (SD=$16,400). Most (86%) of the participants were currently employed, 75% married or partnered, 16% divorced or separated, and 8% never married or partnered. Women described themselves as follows: 5% African American, 8% Asian American, 87% Caucasian. As seen in Table 1, women who were included in these analyses compared to those who were ineligible were slightly younger, had higher incomes, and were more likely to be Caucasian than those who were not included.

Measures

The measures used for this study included overnight urinary cortisol levels, menopause-related factors (MT stage, urinary estrone glucuronide, testosterone, and FSH). In addition, stress-related factors (epinephrine, norepinephrine, perceived stress), social factors (social support, income adequacy, employment, role burden, history of sexual abuse, and parenting), symptoms (hot flashes, mood, memory and sleep) and health-related factors (perceived health, appraisal of physical changes related to aging, BMI, smoking).

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 each month 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. The pooled controls were obtained from 10 healthy female volunteers whose ages correlated with the same age distribution of the study participants. In addition, a sample obtained from a component of the standard curve was placed after every ten unknowns in order to monitor assay drift during each assay run and to monitor variance of replicates. In general, all samples from a calendar year were assayed during the next calendar year and multiple samples from each participant were assayed in the same batch during each year. 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 in relation to creatinine. Urine specimens were assayed for creatinine using the method of Jaffe[25].

Urinary Cortisol—Urine cortisol levels were determined by radioimmunoassay using Coat-A-Count Cortisol Kit (Siemens Medical Solutions, Los Angeles, CA). Coat-A-Count Cortisol is designed for the quantitative measurement of unbound cortisol (hydrocortisone, Compound F) in serum, urine, and heparinized plasma. The assay is highly specific for cortisol and has extremely low cross-reactivity with other steroids, except for prednisolone[26]. All subjects using prednisone or prednisolone were excluded from analysis. The protocol for extraction of cortisol from urine provided by Siemens was followed: urine samples were mixed with dichloromethane (DCM), centrifuged to separate the two phases, the aqueous phase was then removed by aspiration. One hundred microliters of organic phase containing cortisol was transferred into tubes coated with an anti-cortisol antibody. The tubes were evaporated to complete dryness using a gentle stream of nitrogen. Twenty-five microliters of the Zero Calibrator (Siemens Medical Solutions) was introduced to the tubes to rehydrate the evaporated samples. One milliliter of 125-iodine labeled cortisol was then introduced for 45 minute incubation at 37C. The unbound 125-iodine labeled cortisol was decanted, and the bound fraction measured using Cobra Series Auto-Gamma Counting System. Results were determined by interpolation from a logit-log representation of the calibration curve. The curve was established using six calibration standards ranging from 0 to 50 micrograms of cortisol per deciliter. Non-specific binding was subtracted from all tubes. Standards ranging from 5 to 50ug/dL were used throughout the study to calculate variances.

In our laboratory the reporting range for this urinary cortisol assay is 1 to 50 ug/dl, the minimum detectable concentration is 0.2 ug/dL. Inter-assay precision was calculated for each of three samples from the results of 20 extractions each. The coefficients of variation (inter-assay) ranged from 8.2% to 12.5% for samples ranging from 0.9 to 8.3 ug/dL. The intra-assay coefficient of variation was 4.6% (N=376) using a pooled in-house control (3.6ug/dl). There were no significant differences in cortisol values when we compared the DCM extraction to values obtained with an additional chromatographic purification step using a disposable SepPak C-18 cartridge, after initial DCM extraction. Recovery rates ranged from 88.4 to 96.5% when samples were spiked with three cortisol solutions of 5, 10, and 20 ug/dlL.

Menopausal transition-related Factors—MT-related factors included urinary estrone glucuronide, testosterone, and urinary FSH, as well as menopausal transition stage and hot flash severity.

Urinary estrone glucuronide (E1G): Urinary estrone glucuronide was selected as a marker for estrogen because it is stable, can be reliably measured without special preparation, and is highly correlated with serum estradiol levels.[27–29] Urinary E1G was measured by a competitive enzyme immunoassay (EIA) that cross-reacts 83% with estradiol glucuronide, thus measuring both estradiol and estrone in glucuronide forms.[29] Baker and colleagues[30] found that 17<beta>-estradiol-3-glucuronide is excreted in urine across the menstrual cycle in the same pattern as E1G with a clear mid-cycle peak but at concentrations 5 times lower than E1G. The E1G assay developed by O'Connor and colleagues captures meaningful patterns of change with respect to reproductive aging as demonstrated in prior studies of the menopausal transition[31] The assay is described in full elsewhere.[29,32]

All E1G concentrations were adjusted for hydration status using creatinine and corrected for non-parallelism (standardized to a 1:5 dilution) using the methods outlined in O'Connor et al. [29,32] Average E1G concentrations for menopausal and cycling women as measured in our lab were obtained using daily specimens from one full cycle from each of 30 normally cycling U.S. women (N=799 specimens) and 30 daily specimens from 30 post-menopausal U.S. women

(N=892); these specimens were not from the current study. Corrected for dilution factor and non-parallelism, E1G concentrations range from a minimum of 4.688 ng/mL to a maximum of 284.441 ng/mL, with an average of 35.919 ng/mL in cycling women and from 1.879 ng/mL to 44.575 ng/mL with an average of 8.292 ng/mL in menopausal women (unpublished data).

The lower limit of detection of the assay was 3.1 nmol/L. Average recovery from a urine matrix of low, medium and high E1G standard doses was 101%. [29] Intra- and inter-assay CVs were 2.1% and 9.6%, respectively, for an external (BioRad) urine control (mean concentration 2.144 ng/mL); the intra and inter-assay CVs for an internal urine control (mean concentration 1.586 ng/mL) were 2.8% and 14.5%, respectively. 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. The E1G EIA has slight non-parallelism, which is corrected statistically.[29] Measures of urinary E1G in our sample are expressed in ng/mg creatinine.

Urinary FSH—FSH was assayed using Siemens Double Antibody FSH Kit. FSH levels assayed in urine were parallel with serum profiles obtained from reproductive-aged women over the menstrual cycle.[33] The FSH radioimmunoassay is designed for the quantitative measurement of (FSH) in serum and urine. To test for urinary FSH, a 2mL aliquot of each urine sample was pre-treated with an extraction solvent (~1M sodium acetate/acetic acid), followed by overnight incubation in cold acetone. The resulting precipitate was then resuspended in a solution of calibrator matrix prior to assay. To test for fidelity of recovery using the extraction protocols, several calibrator standards obtained from the kit were used, each containing a known amount of FSH in a matrix solution. Recoveries for extraction using this method were on the order of 95%. The protocol for urinary extraction of FSH described in the product insert was followed. In our laboratory the reporting range for urine FSH is 2.0 to100 mIU/mL, the minimum detectable concentration is 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 Siemens Total Testosterone Kit, a solid-phase RIA using a testosterone- specific antibody immobilized to the wall of a polypropylene tube. 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. The calibration curve was prepared using standards ranging from zero to 400ng/dl. In order to assess the validity of the hydrolysis extraction of urine testosterone, we used several calibrator standards obtained from the kit in order to calculate the efficiency of recovery. Standards ranging from 25 to 400 ng/dL were used. The average recovery was 92.7% and ranged from 86.1 to 106%. The inter-assay variation was 12.38% (N=791) and the intra-assay variation (within run) was 8.75%. T levels used in the analysis were reported as ng testosterone per mg creatinine.

Menopausal transition stages were classified from menstrual calendar data. Women not taking any hormones were classified according to stages of reproductive aging: late reproductive, early menopausal transition, late menopausal transition, or early postmenopause, based on staging criteria developed by Mitchell, Woods and Mariella.[6] The names of stages match those recommended at the Stages of Reproductive Aging Workshop (STRAW).[34] The time before the onset of persistent menstrual irregularity during midlife was labeled the late reproductive

stage when cycles were regular. Early MT stage was defined as persistent irregularity of more than 6 days absolute difference between any two consecutive menstrual cycles during the calendar year, with no skipped periods. Late MT stage was defined as persistent skipping of one or more menstrual periods. A skipped period was defined as double the modal cycle length or more for the calendar year. In the absence of a modal cycle length, a population-based cycle length of 29 days was used.[35] Persistence meant the event, irregular cycle or skipped period, occurred one or more times in the subsequent 12 months. Final menstrual period (FMP) was identified retrospectively after one year of amenorrhea without any known explanation. The date of the FMP was synonymous with the term menopause. Early postmenopause (PM) was within five years of the FMP. The staging method has been validated against data from other population-based studies.[36–38]

Diary data—Each woman who collected urine specimens also kept a 3-day health diary on the day before, during and after urine collection. The health diary was the source of the data about intensity of symptoms (hot flashes, mood, cognitive, sleep disturbance), stress levels, and smoking status and perceived health. Data from the diary were aggregated across the three days and were selected to coincide with the dates of the urine specimens.

Symptoms including hot flashes, depressed mood, sleep (problem going to sleep, awakening during the night, early morning awakening) and cognitive (forgetfulness, problem concentrating) symptoms were measured in the 3-day diary. Women rated each symptom on a scale from 0 (not present) to 4 (extreme) for the past 24 hours.[39]

Perceived stress indicators included in the diary assessed a global estimate of stress and several specific areas of stress, including work, parenting, and relationship stress. A global estimate of stress was obtained daily from the question “How stressful was your day?” and rated on a 0 to 6 scale, where 0 was not at all and 6 was extremely stressful. The specific areas of stress included ratings of stress levels related to women’s relationships with others (not children), their role as a parent, and their job or school (using a 0 to 6 scale). Brantly and colleagues found that a global stress rating and the sum of stress ratings across multiple dimensions were significantly correlated.[40]

Women rated their health behaviors in the diary, including the number of cigarettes smoked, and their perceived health status. These data were aggregated to coincide with the date of the urine sample assayed.

In addition to the markers of perceived stress obtained from the diary, we assayed epinephrine and norepinephrine by HPLC after extraction on Bio-Rex cation exchange resin (Bio-Rad) followed by aluminum oxide (Bio-Rad) precipitation using a modification of the LCEC Application Note No. 15 (Bioanal. Syst., 1982). Briefly, a 500uL sample of urine is treated with phosphate buffer prior to loading onto the resin bed, and is then neutralized with distilled water. After treatment with 0.7 M sulfuric acid, the catecholamines are extracted from the resin and onto a matrix of powdered alumina using a solution of 2M ammonium sulfate. After a 10-minute mixing period, the products are extracted with perchloric acid. This eluant is then filtered and then injected onto a Microsorb C-18 column (Rainin) using an autosampler and analyzed with a Coulochem II electrochemical detector (ESA, Inc.). Data acquisition is handled by EZCHROM software (Scientific Software, Inc.). An internal standard, 3,4-dihydroxybenzylamine (DHBA) is mixed with all standards and unknowns prior to extraction. All data are quantitated on peak area ratios, using a standard curve generated in each batch run. The intra-assay variation is 4.7% and the inter-assay variation is 7.85%.

Additional indicators of stress included role burden, employment, parenting, and history of sexual abuse. These were assessed in the annual health update.

Role burden was assessed using the Objective Burden Scale, a 9-item scale that asked women to rate items such as the amount of time they have to themselves and the amount of privacy they have. The ratings were made on a 5 point scale where 1 indicated that their situation was a lot worse and 5 a lot better than 12 months ago. The scale was developed to assess the burden associated with caregiving for elderly relatives and associated with indicators of caregiving tasks that confined the caregiver either temporally or geographically.[41] For the Seattle Midlife Women's Health Study, the stem was: considering all of your different roles and functions in life and how you usually spend your time, tell me the amount each of the following areas in your life has changed from one year ago. Alpha levels range from.71 to .81 over all years in the SMWHS.

Women reported their employment in the annual health questionnaire. Employment was coded as 1 for yes and 0 for no. Women reported number of live births which was used as a marker for parenting status in the annual health questionnaire. This variable was coded as 1 for yes and 0 for no in the analyses reported here.

Sexual abuse history was assessed by asking "Have you ever been sexually assaulted, abused, or molested?" These data were obtained in 2000 and 2001. Also, beginning in 1996 and through the end of the study, we asked "during the past year did you experience any sexual abuse or sexual assault?" A cumulative variable was created to represent any history of sexual abuse or assault and coded as 1 for yes and 0 for no.

Social Factors—Social factors were assessed in the annual health update and included social support and income adequacy. Social support was measured with a 6-item inventory covering ways that people provide support (being able to talk to someone about very personal and private matters, depend on people to lend or give $50, get important advice from others, receive time and energy from others to help take care of something, and get together with people for fun and relaxation). These areas were adapted form Barerra's Arizona Social Support Inventory.[42] Instead of eliciting numbers of people in each area who might and actually did provide each type of support, this adapted version asks about current support availability for each area rated from 0 (not at all) to 3 (quite a bit). Cronbach's alpha for internal consistency reliability in this study was from 0.73 to 0.83 from 1997 to 2005.

Income adequacy was measured using the Income Adequacy Scale developed by Lobo.[43] This scale assesses perceived adequacy of income for six areas of life: daily living, rent or mortgage, food bills, health care, recreation, and child care costs. Participants rated income as more than adequate (4) to not at all adequate (1). Ratings were averaged for a summary score. Cronbach's alpha levels were high (.88 to .95) over the course of the SMWHS.

Health-related Factors included perceived health rating, appraisal of physical changes related to aging, and depressed mood assessed in the annual health report and BMI and smoking, assessed in the diary. Perceived health was measured in the diary with an item asking women to rate their current health on a scale ranging from poor (0) to excellent (10).

Appraisal of physical changes was measured using the Aging Symptoms Inventory (ASI), originally developed to assess health among midlife women. ASI scores were found to be one of the best predictors of emotional closeness in relationships with adolescent children and maternal stress and exhaustion. Women responded to 9 items, including eyesight and shape of the body, rating themselves at the present time on a 10 point scale in which 0 was poor and 10 was excellent.[44] Alpha levels ranged from .64 to .83 over the course of the SMWHS.

Depressed mood with measured with the 20-item version of the Center for Epidemiologic Studies Depression Scale. Depressive symptoms in this scale were derived from clinical criteria

for major depressive disorder (MDD). Categories included positive affect, negative affect, somatic, and interpersonal symptoms [45]. Women rate how often they had a specific feeling during the past week on a 4-point scale from 0 [rarely or none of the time (less than 1 day)] to 3 [most or all of the time (5–7 days)]. The four items of the positive affect category are worded positively and are reverse coded for computation of total score. The total CES-D score is a sum of the ratings of the 20 items, ranging from 0 to 60. A cut-off of 16 has been used to differentiate depressed from non-depressed individuals. In Radloff's study[45], test-retest reliability correlations were .40 or above, and internal consistency reliability as measured by Cronbach's α was .80 or above. In the SMWHS study, α was .88 for the 508 women. The CES-D was administered annually for 15 years from 1990–2005.[9]

Body mass index (BMI) was calculated from self-report of weight and height in the annual health updates using the formula $weight_{kg}/height_{m}^{2}$.

Analyses

Mixed effects modeling using the R library [46–50] was used to investigate whether age, variables related to the menopausal transition, symptoms, stress-related factors, and social and health-related factors were significant predictors of cortisol levels over time. Age was centered at the sample mean to aid in interpretability. Details of the models described briefly below are provided in the appendix.

The initial series of models tested age as a predictor of cortisol. The first model postulated that overall levels of cortisol could differ from woman to woman (random intercept), but would change with age in a common manner (fixed slope). The second model extended the first to postulate a random slope for each woman. The best fitting model for each covariate with age was assessed by using maximum likelihood estimation with Akaike Information Criterion[50]. When the best fitting model was found we extended that model by adding covariates iteratively to test the effect on perceived stress scores over time. Table 2 contains the estimates for the mixed model: random and fixed components. The B_1, B_2, and B_3 estimates are regression parameters based on within-woman variation. B_1 is the mean cortisol level over all ages (intercept), B_2 the amount age predicts the amount age predicts the outcome variable (slope), and B_3 the amount the covariate increases or decreases the outcome variable. The random effects (σ_1, σ_2, and σ_3) represent the unexplained population variances related to the intercept, slope, and residual, respectively.

Each covariate, including stage, was initially added independently to the best fitting model. Finally, all covariates that significantly improved the model fit to the data when entered individually were added simultaneously into a final model. A p-value of .05 was used as the criterion for significance. Different numbers of women and observations occurred with each variable tested because the analysis required pairing of observations of the outcome and predictor variables.

Data from covariates in the diary were matched within 6 days. Health report data were matched to the closest date to the assay data. No data from women using oral or inhaled corticosteroids or estrogen or testosterone preparations were included in these analyses.

Results

To enhance interpretability of results, age was centered at 48.4 years, the mean for the sample included in these analyses. The random intercept and random slope model for age proved to be a better fit for the data (p <0.001) than a model using fixed slope. Although there was a slight non-significant increase in average cortisol levels as women aged (beta=0.004, p=.23),

it was better to allow for an individual rate of change for each woman rather than to assume that there was a single rate of change that held for all women.

We also tested a model using MT stage as a measure of time, but this model failed to converge. (See Figure 1 for an illustration of cortisol levels according to time from the final menstrual period and Figure 2 for cortisol levels according to menopausal transition stages) Cortisol levels (M, SD) for the early menopausal transition stage were 45.3ng/mg creatinine, SD=22.5; for the late MT 53.4, SD=29.6; and for early postmenopause 46.4, SD=25.9) (See Table 2). When added as covariates with age to a model to predict cortisol levels, the menopausal transition-related factors, including urinary estrone glucuronide, testosterone, and FSH were significantly associated with overnight cortisol levels (betas = 0.17, 0.35, 0.07, respectively, $p<0.0001$ for all) (see Table 3). When early and late MT and early PM stages were added as covariates to a model with age, the model did not converge.

Although hot flash severity was a significant covariate with age in predicting cortisol levels (beta = −0.02, $p=0.01$), the beta and p values were small in contrast to those for E1G, FSH, and testosterone. None of the sleep symptoms was a significant covariate. (See Table 3). Likewise, depressed mood, forgetfulness and difficulty concentrating were not significant covariates.

Stress-related factors were significant predictors of overnight cortisol levels. Both epinephrine and norepinephrine were significantly associated with cortisol (beta=0.02, 0.15 respectively, both $p<.0001$). Perceived stress was not significantly related to overnight cortisol levels.

In contrast to findings for MT factors, social factors were not predictive of cortisol levels. Social support, employment, income adequacy, role burden, history of sexual abuse, and health-related factors including appraisal of aging changes, perceived health, depressed mood, and BMI were not significant covariates when considered with age.

When each of the significant covariates was included together in a model with age, estrone, testosterone, and FSH together were significant covariates (See Table 4). The model of overnight cortisol during the MT and early PM was dominated by MT-related endocrine factors.

Discussion

Although cortisol plays an important role in mobilizing responses to stress, overnight cortisol levels in SMWHS women experiencing the MT and early PM were associated significantly with estrone, testosterone, and FSH levels. Cortisol levels were also significantly related to epinephrine and norepinephrine levels, but overnight cortisol levels were related neither to perceived stress and other social stressors nor to severity of symptoms.

These findings suggest that overnight cortisol levels during the menopausal transition and early postmenopause are more likely to be a function of the biological milieu rather than the response to the social milieu. As women approach menopause, increasing levels of FSH stimulate ovarian follicles to produce estrogen, and in response to FSH stimulation, hyperestrogenemia may occur.[51] Estrogen appears to regulate human CRH gene expression, resulting in elevated cortisol levels.[11] Also estrogen levels have been associated with elevated cortisol levels in studies of women who are using oral estrogen therapy. Oral estrogens increase total cortisol levels by increasing circulating cortisol binding globulin levels, an effect not observed with use of transdermal estrogen.[12,13] Also when estrogen-progestogen therapy is administered to premenopausal women, night-time cortisol levels are elevated.[14] Cortisol is produced not only by the adrenal, but also generated in adipose tissue by conversion of inactive cortisone by 11

beta-hydroxysteroid dehydrogenase type 1 (11 beta HSD1) and estrogen has the capacity to up-regulate 11betaHSD1 mRNA expression in preadipocytes from women.[15]

Overnight cortisol levels were positively and significantly related to FSH as well as estrone levels, when considered as covariates with age. In a study of the relationship of first morning urinary cortisol levels and gonadotropins among reproductive aged cycling women, both FSH and LH were positively associated with urinary cortisol levels during both the follicular and the luteal phases of the menstrual cycle.[52] Although overnight urinary cortisol levels were positively related to testosterone levels in the Seattle sample, this relationship has not been reported in other studies of women in the menopausal transition.

The relationship of overnight FSH, estrone and testosterone to cortisol levels also may reflect the changing central control of circadian rhythms during the menopausal transition. During the menopausal transition, when there is perturbation in GnRH pulses and consequent perturbation in the FSH pulses, one sees periods of dramatic change in FSH and estrogen levels.[51] Estrogen levels, in turn, may influence cortisol levels as indicated above. However, a recent study of day night differences in cortisol, thyroid stimulating hormone (TSH), FSH, luteinizing hormone (LH) and free alpha subunit (FAS) in younger and older postmenopausal women under highly controlled environmental conditions indicates that robust day-night differences in cortisol and TSH rhythms exist despite the absence of a circadian rhythm of gonadotropin and FAS levels. These findings suggest differential regulation of these hormonal axes by the suprachiasmatic nucleus (SCN).[53]

Studies of women experiencing hot flashes in laboratory situations indicate that a cortisol rise follows hot flashes.[23] Moreover, women who experienced a cortisol rise from the early to late MTS in the SMWHS cohort had significantly more severe hot flashes than women without the cortisol rise.[5] Of interest is that the analyses reported here, spanning the menopausal transition and early postmenopause, indicate a slight negative association between overnight cortisol levels and hot flashes experienced on the same day or the following day. Laboratory studies of sleep symptoms, hot flashes and cortisol have focused on events occurring within the same time period, typically the same night. Our data reflect hot flash activity during three 24 hour periods surrounding the urine sample assayed for endocrine levels, affording a less precisely timed association than possible with laboratory studies.

The lack of relationship between overnight cortisol levels and sleep symptoms is also puzzling. Nonetheless, results of prior studies with women older than 55 years (mean 69.6 years) indicate that higher 24 hour urinary free cortisol levels were associated with less REM sleep and an earlier rise time in postmenopausal women not using hormone therapy. Moreover, when women were stressed, higher cortisol levels were associated with reduced minutes of sleep in stages 2,3, and 4.[54] Sleep studies using polysomnographic recording reveal great disparity between older women's perceived and recorded sleep.[55,56] It is possible that the sleep measures used in analyses reported here were not sufficiently sensitive to capture the relationships seen with polysomnographic sleep measures.

Both overnight epinephrine and norepinephrine, indicators of autonomic nervous system arousal, were significantly related to cortisol levels. Given the role of cortisol in response to stress, it is surprising that none of the social factors such as income adequacy or role burden was related to overnight cortisol levels, despite these factors being related to perceived stress in our earlier analyses.[57] Moreover, perceived stress was not related to the overnight cortisol levels. Although in our earlier analyses employment was strongly associated with perceived stress, employment was not associated with overnight cortisol levels.[57] In a recently reported study of middle aged employed women, early morning serumcortisol levels were positively related to high demands and low social support.[58] In addition, women in that study who had

been away from work due to illness had higher morning serum cortisol levels than those who had not.

Our findings may reflect the different meaning of overnight urinary cortisol levels compared to a response to a stressful stimulus. A recent assessment of cortisol as a physiologic marker of chronic stress in which women who were stressed by divorce and women who remained married were compared revealed that although overnight urinary free cortisol levels did not differentiate the groups, the stressed group had higher salivary cortisol levels obtained at 6 pm and 9 pm. Likewise, dexamethasone suppression was more effective in the non-stressed women when they were studied on waking 20 minutes later, and at noon.[16]

Overnight urinary cortisol levels probably do not reflect the dynamic response to stressors seen in studies in which cortisol awakening responses (measures taken at awakening and 30 minutes later) reveal increases in cortisol levels in response to stressful life conditions. In studies using salivary cortisol measures obtained on awakening and 30 minutes later, cortisol increases after awakening were associated with work vs weekend days and with lower socioeconomic status.[59] A pattern of lower evening cortisol levels and blunted cortisol amplitude and mesor has been revealed in studies of women with depression.[60] Given the robust persistence of the day-night cortisol rhythm discussed earlier, study of the 24 hour cortisol rhythm will be required to resolve the question of whether blunted circadian amplitude in the cortisol rhythm occurs during the menopausal transition and under stressful conditions. A blunted cortisol amplitude also may reflect the decreasing levels and variability of estrogen as gonadal steroids stabilize across the menopausal transition.

Taken together, these findings are consistent with studies of cortisol during the menopausal transition and midlife, indicating that cortisol levels rise slightly with age. Moreover, overnight cortisol levels are associated with overnight urinary FSH, testosterone, and estrone levels, as well as epinephrine and norepinephrine. Further examination of daytime and night-time patterns of cortisol is necessary to fully understand the relationship of cortisol and vasomotor and sleep symptoms during the MT. The lack of association between social indicators and cortisol levels suggests that overnight cortisol levels do not reflect response to stressors although they are correlated with other biological indicators of stress response, e.g. epinephrine and norepinephrine. Dynamic measures, such as the cortisol awakening response, may be more appropriately used in future stress studies.

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Appendix

APPENDIX*

Let y_{ij} represent the jth cortisol score obtained from the ith woman, where $i = 1,\ldots, M$ and $j = 1, \ldots, n_i$. Here $M = 132$ is the total number of women who have at least one cortisol score in one of the four stages, and n_i is the total number of cortisol scores for the ith wo/man (while $n_i \geq 1$ for all i, the value of n_i varies from woman to woman). Let x_{ij} represent the corresponding age for the woman when the value y_{ij} was recorded. In the models below, x_{ij} is centered at 48.4, which is the approximate sample mean of all recorded ages.

The first age-based model assumes that

$$y_{ij}=\beta_1+b_{1,i}+\beta_2\,(x_{ij}-48.4)+\varepsilon_{ij}, \quad (1)$$

where the fixed effect β_1 represents the mean cortisol score at age 48.4 over the population of women; $b_{1,i}$ is a random variable (RV) that is normally distributed with mean zero and variance

σ^2_1 (this RV represents the deviation from β_1 for the ith woman); β_2 is a fixed slope; and ε_{ij} represents the error terms, which are independent and normally distributed with mean zero and variance σ^2_ε (the RVs $b_{1,i}$ and ε_{ij} are assumed to be independent of each other). This model basically postulates that, while the overall levels of cortisol scores can differ from woman to woman, the scores change with age in a common manner.

The second model differs from the first in that it postulates a random slope for each woman:

$$y_{ij}=\beta_1+b_{1,i}+b_{2,i}\,(x_{ij}-48.4)+\varepsilon_{ij}, \tag{2}$$

where β_1, $b_{1,i}$ and ε_{ij} are interpreted in the same manner as the first model, while $b_{2,i}$ is an RV representing the slope associated with the ith woman. We assume that $b_{2,i}$ is normally distributed with mean β_2 and variance σ^2_2. The RVs $b_{1,i}$ and ε_{ij} are assumed to be independent of each other, as are $b_{2,i}$ and ε_{ij}; however, $b_{1,i}$ and $b_{2,i}$ are allowed to be correlated. This model basically postulates both the overall levels of cortisol scores and their slopes can differ from woman to woman.

The third model extends the second by adding one or more covariates. In the case of a single covariate z_{ij}, the model takes the form

$$y_{ij}=\beta_1+b_{1,i}+b_{2,i}\,(x_{ij}-48.4)+\beta_{3,i}z_{ij}+\varepsilon_{ij}, \tag{3}$$

where $\beta_{3,i}$ is a fixed effect associated with z_{ij}. Additional covariates are added in an obvious way.

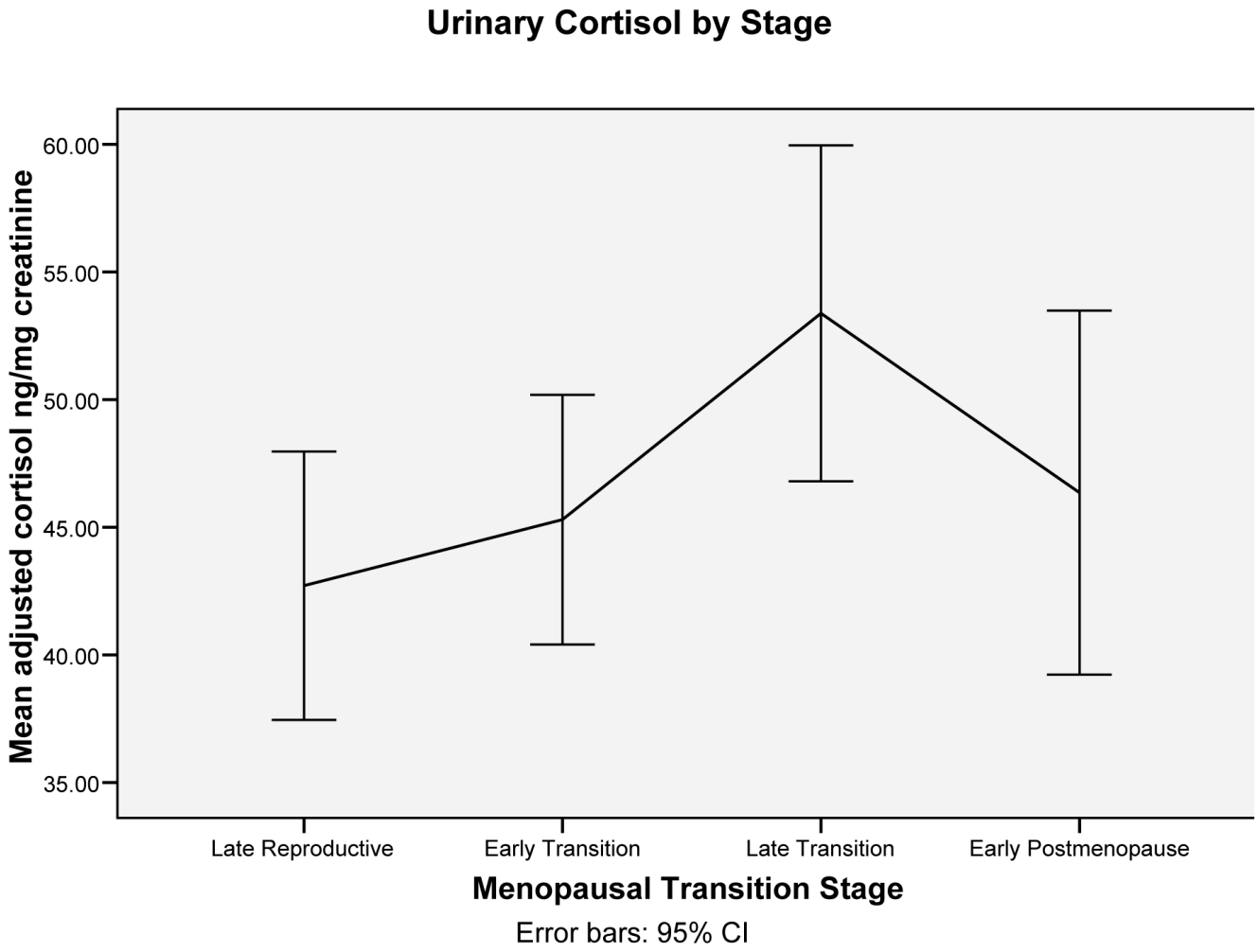

Figure 1.
Cortisol Levels by Years Since Final Menstrual Period (FMP)

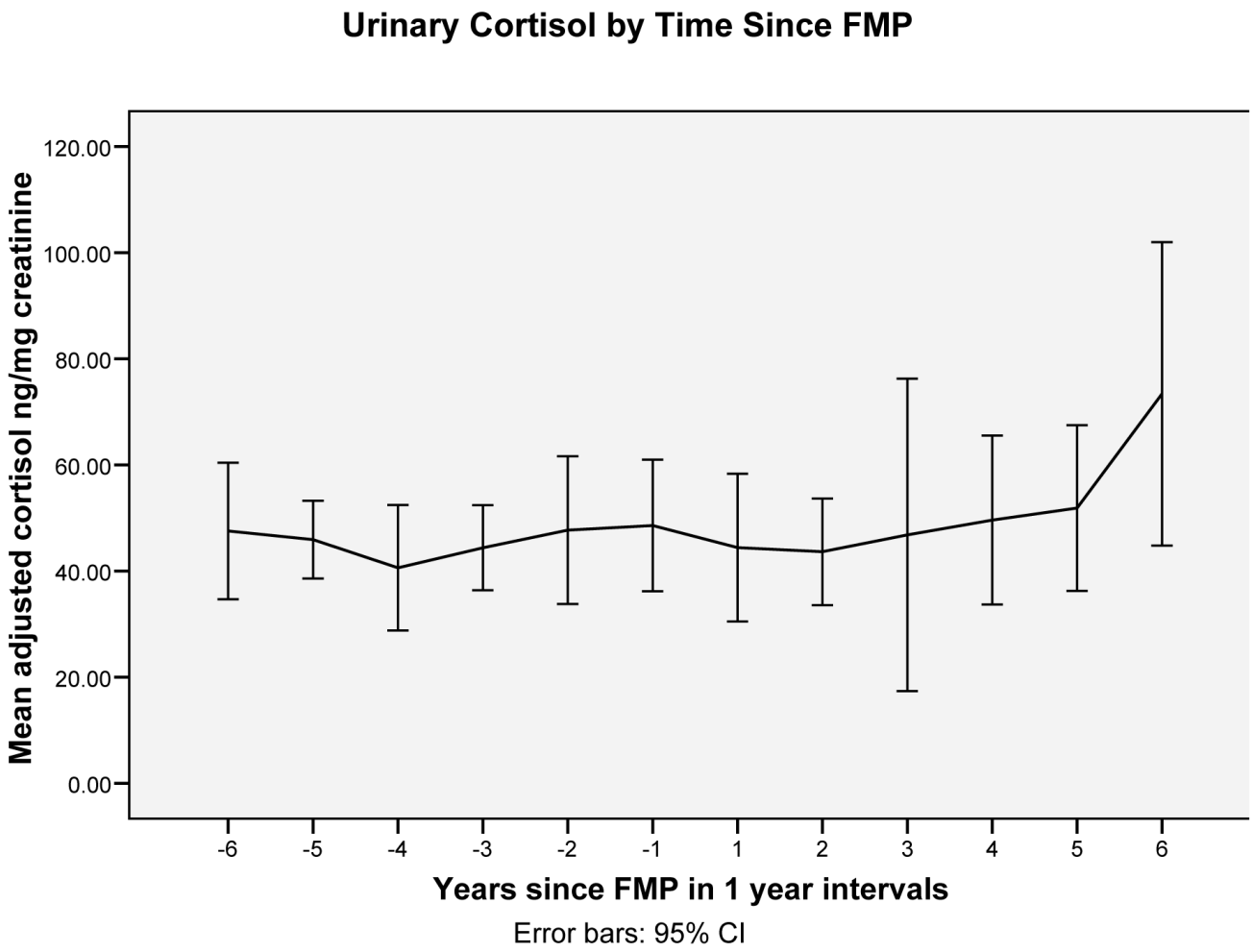

Figure 2.
Cortisol Levels by Menopausal Transition Stage

Table 1

Sample Characteristics of the Eligible and Ineligible Women in the Mixed Effects Modeling Analyses of Cortisol Level at Start of Study (1990–1991).

Characteristic	Eligible Women (n=132) Mean (SD)	Ineligible Women (n=376) Mean (SD)	*p* value*
Age (years)	40.4 (3.9)	42.1 (4.8)	<0.0001
Years of education	16.0 (2.8)	15.5 (2.9)	0.09
Family income ($)	41,400 (16,400)	38,000 (18,600)	0.02
Characteristic	**N (Percent of Eligibles)**	**N (Percent of Ineligibles)**	***p* value****
Currently employed			0.96
Yes	114 (86.4)	324 (86.2)	
No	18 (13.6)	52 (13.8)	
Race/ethnicity			0.003
African American	7 (5.3)	51 (13.6)	
Asian/Pacific Islander	10 (7.5)	33 (8.8)	
Caucasian	115 (87.1)	276 (73.4)	
Other (Hispanic, Mixed)	0 (0)	16 (4.2)	
Marital Status			0.17
Married/partnered	99 (75.0)	249 (66.2)	
Divorced/widowed	21 (15.9)	95 (25.3)	
Never married	10 (7.6)	25 (6.6)	

* Independent t-test

** Chi-square test

Table 2

Urinary Cortisol, Estrone Glucuronide, FSH, and Testosterone Levels (M, SD) according to Menopausal Transition Stages*.

Endocrine	**Late Reproductive Stage (n=56)**	**Early Menopausal Transition (n=84)**	**Late Menopausal Transition (n=80)**	**Early Postmenopause (n=53)**
Cortisol (ng/mg creatinine)	42.7 (19.6)	45.3 (22.5)	53.4 (29.6)	46.4 (25.9)
Estrone glucuronide (ng/mg creatinine)	21.1 (10.0)	25.8 (19.0)	25.3 (12.2)	13.9 (6.3)
FSH mIU/mL	14.1 (5.4)	18.3 (11.9)	34.5 (24.7)	56.4 (31.8)
Testosterone** ng/mg creatinine	20.7 (10.3) n=53	22.3 (12.9) n=82	22.4 (11.5) n=78	23.6 (11.5) n=51

* excluding values for women using corticosteroids

** excluding values for women using androgen medications

Table 3

Random Effects Models for Cortisol Levels (β_1) with Age as Predictor (β_2) and with Individual Covariates (β_3)

	Mean Values (*p* values)			Standard Deviations			Number	
Predictor	**β_1***	**β_2***	**β_3***	**σ_1****	**σ_2****	**σ_ε****	**Women**	**Observations**
Age (48.4)	1.51 (<0.0001)	.004 (0.23)	-	0.18	0.02	0.36	132	5218
Menopause- related factors								
Hot flash severity	1.51 (<0.0001)	0.006 (0.08)	−0.02 (0.01)	0.16	0.02.	0.36	130	4465
Estrone glucuronide ($\log_{10}$) (1.2)	1.50 (<0.0001)	0.004 (0.16)	0.17 (<0.0001)	0.18	0.02	0.36	132	5126
FSH ($\log_{10}$) (1.1)	1.50 (<0.0001)	0.001 (0.75)	0.07 <0.0001)	0.17	0.02	0.36	132	5218
Testosterone ($\log_{10}$) (1.3)	1.51 (<0.0001)	0.003 (0.30)	0.35 <0.0001)	0.18	0.02	0.35	132	5096
Stress related factors								
Epinephrine ($\log_{10}$) (0.4)	1.53 (<0.0001)	0.002 (0.47)	0.20 <0.0001)	0.15	0.02	0.36	132	2086
Norepinephrine ($\log_{10}$) (0.4)	1.53 (<0.0001)	0.003 (0.39)	0.15 0.0001)	0.16	0.01	0.37	132	2086
Perceived stress	1.50 (<.0001)	<0.01 (.22)	<.001 (.90)	0.16	0.02	0.36	130	4465
Symptoms								
Depressed mood	1.50 (<0.0001)	0.004 (0.22)	0.001 (0.94)	0.16	0.02	0.36	130	4465
Awakening during night	1.50 (<0.0001)	0.004 (0.24)	0.005 (0.56)	0.16	0.02	0.36	130	4465
Early morning awakening	1.50 (<0.0001)	0.004 (0.22)	−0.001 (0.88)	0.16	0.02	0.36	130	4465
Problem going to sleep	1.51 (<0.0001)	0.004 (0.22)	−0.02 (0.06)	0.16	0.02	0.36	130	4465
Forgetfulness	1.50 (<0.0001)	0.004 (0.23)	0.006 (0.60)	0.16	0.02	0.36	130	4465
Problem concentrating	1.50 (<0.0001)	0.004 (0.22)	0.008 (0.47)	0.16	0.02	0.36	130	4465
Social factors								
Parenting (Number live births)	1.50 (<0.0001)	0.004 (0.25)	0.004 (0.73)	0.18	0.02	0.36	132	5218

	Mean Values (*p* values)			Standard Deviations			Number	
Predictor	**β_1***	**β_2***	**β_3***	**σ_1****	**σ_2****	**σ_ε****	**Women**	**Observations**
Social support	1.46 (<0.0001)	0.003 (.47)	0.02 (.55)	0.07	0.01	0.33	127	544
Employed	1.57 (<0.0001)	0.003 (0.29)	−0.07 (0.06)	0.18	0.02	0.36	132	5218
History sexual abuse	1.52 (<0.0001)	0.004 (0.22)	−0.05 (0.16)	0.18	0.02	0.36	132	5218
Income adequacy	1.42 (<0.0001)	0.003 (0.49)	0.03 (0.17)	0.08	0.01	0.33	127	587
Role burden	1.45 (<0.001)	<0.001 (0.41)	0.02 (0.49)	0.09	0.14	0.33	127	590
Health-related factors								
Depression score (CESD)	1.53 (<.0001)	.002 (0.50)	−0.002 (0.16)	0.08	0.01	0.33	127	589
Perceived health	1.46 (<0.0001)	0.004 (0.26)	0.01 (0.14)	0.16	0.02	0.36	130	4465
Physical appraisal	1.46 (<.0001)	.003 (0.47)	.008 (0.44)	0.08	0.01	0.33	127	590
BMI (26.1)	1.50 (<0.0001)	0.004 (0.19)	−0.002 (0.36)	0.18	0.02	0.36	132	5218
Smoker	1.50 (<0.0001)	0.004 (0.21)	0.03 (0.54)	0.18	0.02	0.36	132	5218

*β_1, β_2, β_3 are the fixed effects (group averages) for the intercept, slope and covariate.

**σ_1, σ_2, σ_ε are the random effects (variability) for the intercept, slope and residual error.

Table 4

Final Random Effects Model for Cortisol Levels (β_1) with Age as Predictor (β_2) and Other Significant Covariates Entered Simultaneously
(N=130; observations=4235)

	Coefficient (Betas)	Standard Error/Standard Deviation	*p* value
Fixed effects			
β_1 intercept	1.49	0.02	<0.0001
β_2 Age (48.4) years	0.003	0.003	0.39
β_{logE1G} Estrone (1.2)	0.13	0.02	<0.0001
β_{logFSH} FSH (1.1)	0.07	0.01	<0.0001
β_{Testos} Testosterone (1.3)	0.26	0.02	<0.0001
Random effects			
b_1 Intercept σ_1		0.15	
b_2 Age (48.4) years σ_2		0.03	
b_ε residual σ_ε		0.31	