Published in final edited form as:

Menopause. 2014 June ; 21(6): 646–652. doi:10.1097/GME.0000000000000122.

Endocrine Biomarkers and Symptom Clusters during the Menopausal Transition and Early Postmenopause: Observations from the Seattle Midlife Women's Health Study

Nancy F. Woods, PhD, RN, FAAN¹, Lori A. Cray, PhD, RN², Ellen S. Mitchell, PhD¹, and Jerald R. Herting, PhD¹

¹ School of Nursing, University of Washington, Seattle, WA, United States

² College of Nursing, Seattle University, Seattle, WA, United States.

Abstract

Objective—During the menopausal transition and early postmenopause participants in the Seattle Midlife Women's Health Study (SMWHS) were likely to belong to one of three symptom severity classes: severe hot flashes with moderate sleep, mood, cognitive, and pain symptoms (High-severity Hot Flash); moderate levels of all but hot flashes (Moderate Severity); and low levels of all (Low Severity). We tested models of differential effects of hypothalamic-pituitary-ovarian (HPO), hypothalamic-pituitary-adrenal (HPA), and autonomic nervous system (ANS) biomarkers on the three symptom severity classes.

Methods—SMWHS participants recorded symptoms monthly in diaries and provided overnight urine samples several times per year that were analyzed for estrone, follicle stimulating hormone, cortisol, testosterone, epinephrine and norepinephrine. Multilevel latent class analysis with multinomial regression was used to determine the effects of HPO, HPA, and ANS biomarkers on symptom severity class membership.

Results—Having lower estrogen levels and higher FSH levels were associated significantly with belonging to the High-severity Hot Flash vs the Low Severity class. Having lower epinephrine and higher norepinephrine levels increased the likelihood of belonging to the High-severity Hot Flash vs the Low Severity class. Having lower epinephrine levels was associated significantly with belonging to the Moderate Severity vs the Low severity class. Cortisol and testosterone were unrelated to symptom severity class membership.

Conclusion—Association of HPO biomarkers (estrogen, FSH) with the High-severity Hot Flash class was anticipated based on prior hot flash research and associations of HPA biomarkers were as expected based on earlier laboratory studies. Association of lower epinephrine levels with the Moderate Severity class suggests these symptoms may be mediated by the ANS.

Keywords

menopausal transition; symptom clusters; estrogen; FSH; cortisol; epinephrine; norepinephrine

A majority of women experiencing the menopausal transition (MT) and early postmenopause (PM) report being bothered by hot flashes ¹⁻³ and co-occurring symptoms (symptom clusters). ⁵⁻⁸ Cray and colleagues have identified three symptom severity clusters

(latent classes) women participating in the Seattle Midlife Women's Health Study (SMWHS) experienced, which were differentially associated with stages of reproductive aging.⁹ The most prevalent cluster included low severity hot flashes, mood, sleep, cognitive, and pain symptoms, accounting for approximately 70% of observations. Another cluster accounting for approximately 13% of observations was characterized by high severity hot flashes and moderate severity mood, sleep, cognitive, and pain symptoms and was associated with the late menopausal transition and early postmenopause. The third cluster accounted for 17% of observations and included low severity hot flashes and moderate severity sleep, mood, cognitive and pain symptoms.⁹

Several reports from longitudinal studies of the menopausal transition and early postmenopause support the association between hypothalamic-pituitary-ovarian (HPO) axis functioning and individual symptoms women experience during this period. Recently published results of the Penn Ovarian Aging and SWAN studies indicated that hot flashes were associated with variability in and levels of estradiol and follicle stimulating hormone (FSH) levels.^{5,10} Studies of sleep symptoms during the menopausal transition and postmenopause revealed that lower estradiol levels were associated with night-time awakening,¹¹ and findings from the SWAN study indicated that lower estradiol levels and higher FSH were associated with difficulty falling asleep and remaining asleep.¹² Moreover, these findings were supported in later studies of polysomnographic sleep in a subset of SWAN participants.¹³ In contrast, findings from the Penn Ovarian Aging cohort indicated that poor sleep was unrelated to estradiol, testosterone (T), and FSH.¹⁴

Studies of depressed mood symptoms revealed mixed findings: depressed mood as measured by the CESD was significantly associated with higher testosterone levels among SWAN participants.¹⁵ Among Penn Ovarian Aging participants, depressed mood was associated with increased levels of follicle stimulating hormone (FSH) and leutinizing hormone (LH) and increased variability of estradiol (E2), FSH and LH.¹⁶ Among Seattle Midlife Women's Health Study participants urinary estrone, FSH, and testosterone levels were not associated with CESD scores,¹⁷ consistent with the finding of no association between major depressive disorder and endocrine levels and change among SWAN participants.¹⁸ Cognitive symptoms were unrelated to HPO hormone levels in the SWAN,^{19,20} Seattle Midlife Women's Health Study,²¹ and Penn Ovarian study.²²

Pain symptoms (aches, joint pain and stiffness) have been associated with estrogen levels and variability in the Penn Ovarian Aging population. Aches, joint pain and stiffness were associated with estradiol variability,⁵ but neither joint pain and back pain were associated with urinary levels of estrone or FSH in the SMWHS population.²³

To date there has been little investigation of the role of the neuroendocrine effects associated with the hypothalamic-pituitary-adrenal (HPA) and autonomic nervous system (ANS) on symptoms women experience during the MT and early PM. A rise in cortisol during the late MT stage was found in the SMWHS population,²⁴ but no difference in cortisol levels during the menopausal transition stages and early postmenopause in the SWAN population have been reported.²⁵ Among the SMWHS population, cortisol was positively associated with urinary estrone, epinephrine and norepinephrine levels during the MT stages.²⁶

Early studies of hot flashes in laboratory settings involved inducing hot flashes and measuring endocrine responses. Meldrum and colleagues²⁷ found that ACTH levels rose shortly after women experienced hot flashes and cortisol levels rose about 15 minutes after women experienced hot flashes. Moreover, Freedman and colleagues²⁸ demonstrated associations between brain metabolites of norepinephrine (MHPG) and hot flashes, but not peripheral metabolites of norepinephrine (VMA), suggesting a role for brain levels of norepinephrine and hot flashes. Among the SMWHS population higher cortisol levels were associated with less severe problems getting to sleep, but not night time awakening or early morning awakening; epinephrine and norepinephrine levels were not associated with sleep symptoms.¹¹ Higher cortisol levels were associated with less back pain and higher norepinephrine and epinephrine levels were marginally associated with higher levels of joint pain.²³ Depressed mood was not associated with cortisol, epinephrine or norepinephrine levels,¹⁷ nor were cognitive symptoms of difficulty concentrating and forgetfulness.²¹

The modulation of hypothalamic-pituitary-ovarian axis function by the hypothalamic-pituitary-adrenal axis has been demonstrated in earlier work.²⁹ Although it is likely that during the MT and early PM changes will occur in HPA axis and autonomic functioning when ovarian function is transitioning to a new pattern in which estrogens are increasingly of non-ovarian origin, to date there have been no investigations of HPO, HPA, and autonomic influences on symptoms report during this period, aside from hot flashes.

Data from the SMWHS support women's experience of multiple co-occurring symptoms and their relationship to menopausal transition stages,⁹ but to date researchers have not explored the association between hypothalamic-pituitary-ovarian and hypothalamic-pituitary-adrenal and autonomic nervous system function and symptom severity clusters. The primary approach to understanding the relationship of menopause and symptoms has been motivated by hypothesizing that changes in the HPO axis such as declining estrogen levels and rising FSH are responsible for individual symptoms. Identifying symptom severity clusters and related neuroendocrine mechanisms may serve as the basis for tailoring therapy to co-occurring symptoms characterized by severity levelsexperienced by individual women and suggesting novel approaches to symptom management for women during the MT and early PM. Therefore, the purpose of this study was to test a model hypothesizing differential effects of hypothalamic-pituitary-ovarian (HPO) (urinary estrone, FSH, testosterone), hypothalamic-pituitary-adrenal (HPA) (cortisol), and autonomic nervous system (ANS) (epinephrine, norepinephrine) biomarkers on symptom severity clusters women experience during the MT and early PM.

Methods

Sample and Design

The current analysis used data from the longitudinal Seattle Midlife Women's Health Study (SMWHS) which included participants for as many as 20 years of follow-up. In the original study 508 women were enrolled between 1990 and 1992. After completing an initial in-person interview, 390 women consented to provide data annually by questionnaire, daily menstrual calendar, and/or health diary. At the end of 5 years, 243 women consented to continue to participate for an additional 5 years and 170 agreed to provide a first morning

urine specimen 8–12 times per year on day 6 of the menstrual cycle.^{17,30–31} For the current analysis, the data set contained data from 292 women whose menstrual calendars could be assigned a menopausal transition stage and who provided diary data on at least one occasion. Eligible participants (N=130 of 292, 45%) for this analysis were those who contributed ratings of hot flash, sleep, pain, mood, cognitive and tension symptoms from the health diaries on at least one occasion between 1990 and 2011, were in the late reproductive, early or late menopausal transition stages, or early postmenopause during the course of the study and provided a first morning urine sample on at least one occasion, and for whom assays were available of estrone, FSH, testosterone, cortisol, epinephrine, and norepinephrine. Women whose menstrual calendars were not able to be assigned a menopausal transition stage due to hormone use were excluded. This sample provided data points for a total of 3175 measurement occasions.

Measures

Symptoms—A 3-day health diary included 47 symptoms that were rated for severity over the previous 24 hours on a 5-point Likert-type scale (0 = not present, 4 = extreme)¹⁷. Women completed the diary over three consecutive days each month thru 2000 then completed the diary 4 times per year (quarterly) thereafter. Women filled out the diary on menstrual cycle days 5, 6, and 7 if they were still having periods; if not, on any consistent 3 days each month or each quarter. Ratings were averaged over 3 days.¹⁷ The five symptom groups used in these analyses were determined from a previous principal components analysis of the following fifteen individual symptoms: early awakening, awakening at night, difficulty falling to sleep (sleep); joint pain, backache, headache (pain); depressed, mood changes, crying, irritable (mood); problem concentrating, forgetful (cognitive); nervous, panic, tension (tension).³² Hot flash was used as a single symptom.

Biomarkers—All urinary assays were performed in the University of Washington (UW) Center for Women's Health Research 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 ethylenediaminetetraacetic 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. 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 specimen³³. The methods by which each of these biomarkers were assayed are described in greater detail elsewhere¹⁷.

HPO Biomarkers

Urinary estrone glucuronide (E1G)—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.³⁴⁻³⁶ 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^{37,38} The assay is described in full elsewhere.³⁸ Measures of urinary E1G in our sample are expressed in ng/mg creatinine.

Urinary FSH—FSH was assayed using Siemens Double Antibody FSH radioimmunoassay kit. FSH levels assayed in urine were parallel with serum profiles obtained from reproductive-aged women over the menstrual cycle.⁴⁰ In our laboratory the reporting range for urine FSH is 2.0 to 100 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. 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.

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 prednisolone⁴¹. All participants using prednisone or prednisolone were excluded from analysis.

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).

Urinary epinephrine and norepinephrine—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). The intra-assay variation is 4.7% and the inter-assay variation is 7.85%.

Menopausal transition stages and early postmenopause—Menopausal transition (MT) refers to the period of time leading up to the final menstrual period in which persistent

menstrual irregularity occurs.⁴² Using menstrual calendar data, menstrual patterns for women not taking any hormones were classified according to stages of reproductive aging: late reproductive, early menopausal transition or late menopausal transition based on staging criteria developed by Mitchell, Woods and Mariella⁴³ and validated in later studies.⁴⁴⁻⁴⁶ Early postmenopause (PM) includes the five years after the final menstrual period. The names of stages and the definition of early PM match those recommended at the Stages of Reproductive Aging Workshop (STRAW)⁴² and validated in follow-up studies by the ReSTAGE Collaboration⁴³. The STRAW criteria define the stages as follows: early transition is characterized by a persistence of a seven or more days difference between two consecutive cycle lengths; late transition is characterized by an interval of amenorrhea of at least sixty days and early postmenopause is defined as the first 5 years since the final menstrual period.⁴⁴⁻⁴⁶ The final menstrual period (FMP) was defined as the last day of the final menses, using data for at least one year after this event.

Data Analysis

Multilevel latent class analysis (MLCA) was used to identify empirically latent classes of menopausal symptoms based on individual diary observations, using the five symptom groups identified from a previous principal components analysis and hot flash as an individual symptom. Traditional latent class analysis is a statistical method used to identify subgroups of related cases that are not directly observed based on categorical or continuous observed variables. This traditional method assumes independence whereas MLCA allows for the nested structure of the diary data.⁴⁷ All models use monthly symptom episodes as the unit of measure and account for the clustering of symptom episodes within individual women. The HPO (E1G, FSH, testosterone), HPA (cortisol) and ANS (epinephrine, norepinephrine) biomarkers were included as covariates to assess effect on class membership.

Results

As seen in Table 1, women whose data were available for analysis and were eligible for inclusion were midlife women with a mean age of 48.5 (SD, 4.0) years and 16.1 (SD, 2.5) years of education at the beginning of the study. Most (98.5%) were employed, 76% were married or partnered, 22% were divorced, separated or widowed, and 2% were never married or partnered. Eligible women described themselves as follows: 5.4% African American, 7.7% Asian/Pacific Islander, and 86.9% white. As seen in table 1, the women who were included in this analysis, compared to those who were ineligible, were slightly older and less likely to be a smoker, but similar in respect to employment status, ethnicity, marital status, and education, body mass index (BMI) and exercise activity level.

As in prior analyses, we found 3 classes of symptom severity cluster episodes: high hot flash; low hot flash; and all low symptoms. Class 1 accounted for 13% of the sample and included high hot flash severity and lower severity levels of other symptoms. Class 2 accounted for 17% of the sample and included low severity hot flashes and moderate severity levels of other symptoms. Class 3 accounted for 70% of the sample and included low severity symptoms.

As seen in Table 2, relative to the low severity symptom class (class 3), higher levels of estrogen (OR= 0.016) significantly reduced the likelihood of being in the high hot flash class (class 1) while women with higher levels of FSH (OR= 2.870) were significantly more likely to be in the high hot flash class. Higher levels of epinephrine reduced the likelihood of being in the high hot flash class (OR= <0.001) and higher levels of norepinephrine increased the likelihood of being in this class relative to the low severity symptom class (OR= 10.507). There were no significant effects found for cortisol or testosterone levels.

In addition, epinephrine levels significantly differentiated the low hot flash/moderate severity symptoms cluster (class 2) from the low severity symptoms cluster (class 3) (OR=0.001). Having lower epinephrine levels increased the likelihood of having the low hot flash/moderate severity symptom cluster than the low severity symptom cluster. None of the HPO biomarkers was associated with the low hot flash/moderate symptoms cluster, nor was cortisol or norepinephrine.

Discussion

The analyses reported here assessed the direct effects of HPO (estrogen, FSH, testosterone), HPA (cortisol) and ANS biomarkers (epinephrine, norepinephrine) on symptom severity clusters (latent classes). As we anticipated, the HPO biomarkers, estrogen and FSH had a significant effect on symptom severity cluster membership, with lower levels of estrogen and higher levels of FSH increasing the probability of membership in the high severity hot flash cluster as compared to the all low symptom severity cluster. Testosterone levels and cortisol were not significantly related to cluster membership. Higher levels of the ANS biomarkers, epinephrine and norepinephrine, also had a significant effect on symptom severity cluster membership, with the higher levels of epinephrine reducing the likelihood of belonging to the high hot flash cluster while higher levels of norepinephrine conversely increased the probability. Moreover, epinephrine was the sole biomarker distinguishing membership in the low hot flash/moderate symptom severity cluster from the low symptom severity cluster.

These findings represent the first reports of both HPO and HPA/ANS biomarkers as correlates of symptom severity clusters women experience during the late reproductive through the early postmenopause stages of reproductive aging. Our earlier analyses indicated that the clusters of high severity hot flashes with moderate levels of mood, sleep, cognitive and pain symptoms were more likely to occur during the early and late MT stages and early PM⁹. Thus it is not surprising that this cluster was associated with lower levels of urinary estrone and high FSH levels given the trajectory of these endocrine changes during the MT and early PM.¹⁰ Moreover, the association of hot flashes with sleep, mood, and pain symptoms and with estrogen levels has been established in studies of individual symptoms.^{5,10-17}

The role of cortisol in the high hot flash/moderate symptom severity cluster is puzzling. Analyses of individual symptoms in the SMWHS population have not linked cortisol to individual symptoms, including hot flashes, depressed mood, cognitive, pain, and sleep symptom^{4,11,17,21,23} despite the observed rise in cortisol during the late menopausal

transition stage. The association of cortisol with urinary estrone, FSH, and testosterone in the SMWHS population may suggest that the transition of estrogen production from an ovarian to an adrenal source during the menopausal transition may be marked by a transient period in which levels of both cortisol and HPO axis hormones are elevated.²⁶ Nonetheless, cortisol does not appear associated with any of the symptom clusters in these analyses.

Earlier research focusing on hot flashes has established the relationship between norepinephrine levels and hot flashes, but these investigations did not include other symptoms in their reported analyses.^{27,28} Nonetheless, laboratory investigations have established the link between elevated norepinephrine levels and hot flashes.^{27,28}

Associations of the ANS biomarkers with the low hot flash/moderate sleep, mood, cognitive, and pain symptom severity cluster are of interest. This symptom severity cluster was significantly associated with lower epinephrine levels, but not with norepinephrine or cortisol. Clustering of the sleep, mood, cognitive, and pain symptoms resembles the collection of emotional and somatic symptoms that are often included in measures of depressed mood symptoms. This cluster was not associated with menopausal transition stages and early postmenopause in our earlier analyses, nor is it significantly associated with HPO biomarkers, suggesting that it may be associated with other factors, such as the stressful nature of women's lives or their responses to aging. These explanations require further investigation.

Recent studies of stress reactivity in women revealed that hypoactive responses to stress were associated with depression five years later.⁵² Chronic and repeated stressors may lead to persistent dysregulation of stress responsive systems, which has been characterized as allostatic load.⁵³ Exhaustion of stress responsive systems as consequences of increased allostatic load may manifest in blunted sympathetic nervous system reactivity. Thus dysregulation in stress responsive systems after repeated elevations of stress-related neuroendocrine responses may manifest in the current findings as lower epinephrine levels. Women with histories of abuse exhibit alterations in biological profiles modulated by their menstrual cycles.⁵⁴ Given that both estrogen and progesterone modulate beta adrenergic responses, further research investigating the relationship between the menopausal transition and early postmenopause, HPO endocrine changes, and symptoms such as those included in the cluster with moderate levels of all but hot flashes may contribute to understanding the relationship we described between this cluster and stress response.

The current report includes data from a subset of SMWHS participants and thus is limited to generalizability to a well-educated and predominantly white population. These findings bear replication among a more ethnically diverse population such as those enrolled in SWAN or the Penn Ovarian Aging study.

Conclusion

In summary, we found that levels of urinary estrone, FSH, epinephrine, and norepinephrine differentiated women experiencing a symptom cluster with high severity hot flashes and moderate levels of mood, sleep, cognitive, and pain symptoms from those with low severity

symptoms. We also found that having a low level of epinephrine distinguished women with low severity hot flashes and moderate levels of the other symptoms from women with low severity symptoms during the menopausal transition and early postmenopause. Taken together, these findings suggest that both the HPO axis and ANS function may be influential in symptom clusters women experience during the menopausal transition and early postmenopause.

Acknowledgments

Funding support: This work was supported by grants from the National Institute of Nursing Research (NINR 1R21NR012218-01 Menopause Symptom Clusters: Refocusing Therapeutics; NR 04141 - Menopausal Transition: Biobehavioral Dimensions; P30 NR 04001, P50-NR02323 – Center for Women's Health and Gender Research).

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Table 1

Characteristics of women whose data were eligible for inclusion in the latent class analysis compared with those whose data were not eligible

Characteristic	<u>Eligible Women (n=130)</u> Mean (SD)	<u>Ineligible Women (n=162)</u> Mean (SD)
Age (years)	48.5 (4.0)	44 (4.4) *
Years of education	16.1 (2.5)	15.8 (2.7)
Characteristic	<u>N (Percent)</u>	<u>N (Percent)</u>
Currently employed		
Yes	128 (98.5)	154 (95.1)
No	2 (1.5)	8 (4.9)
Race/ethnicity		
African American	7 (5.4)	13 (8.0)
Asian /Pacific Islander	10 (7.7)	17 (10.5)
White	113 (86.9)	126 (77.8)
Other (Hispanic, Mixed)	0 (0)	6 (3.7)
Marital Status		
Married/partnered	99 (76)	116 (71.7)
Divorced/widowed/not partnered	28 (22.0)	36 (22.1)
Never married/partnered	3 (2)	10 (6.2)
Smoking Status:		
Non-smoker	116 (89.5)	131 (80.7) **
Smoker	14 (10.5)	31 (19.3)
Body Mass Index (mean)	26.8	25.7
Level of Activity (mean # of minutes of exercise per day)	28.9	23.8

* p<.001

** p<.05 - Independent samples t-test with 95% confidence interval

Table 2

Effects of Endocrine (HPO, HPA & ANS) Biomarkers on Latent Class Membership (beta, SE, Wald statistic; Odds Ratio, 95% CI Limits)

Class 1 (n=399)	<i>b</i>	<i>SE</i>	<i>Wald(1)</i>	<i>OR</i>	<i>CI Lower Limit</i>	<i>CI Upper Limit</i>
<i>Intercept</i>	-1.857	0.323	-5.757	NA	NA	NA
EIG	-4.148	1.925	-2.155*	0.016	0.001	0.375
FSH	1.054	0.260	4.053**	2.870	1.871	4.402
TEST	1.027	0.760	1.352	2.793	0.800	9.747
CORT	-0.143	0.177	-0.806	0.867	0.648	1.160
EPI	-11.484	3.702	-3.102 [†]	<0.001	<0.001	0.005
NEPI	2.352	0.750	3.135 [†]	10.507	3.059	36.094

Class 2 (n=547)	<i>b</i>	<i>SE</i>	<i>Wald(1)</i>	<i>OR</i>	<i>CI Lower Limit</i>	<i>CI Upper Limit</i>
<i>Intercept</i>	-1.538	0.280	-5.501	NA	NA	NA
EIG	0.139	0.461	0.301	1.149	0.538	2.453
FSH	-0.200	0.399	-0.501	0.819	0.425	1.578
TEST	0.006	0.573	-0.011	0.994	0.387	2.550
CORT	-0.191	0.187	-1.024	0.826	0.608	1.123
EPI	-6.527	3.148	-2.073*	0.001	<0.001	0.260
NEPI	1.503	0.827	1.817	4.494	1.153	17.514

Low symptomatic group (class 3) was used as the referent (n=2229)

95% Confidence Interval (CI)

* p<.05

** p<.001

[†] p<.01