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Hormone Variability and Hot Flash Experience: Results from the Midlife Women's Health Study

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Abstract

Objective: Hot flashes are believed to be related to hormonal changes. However, the relationship between hormonal fluctuations and hot flashes has not been studied. The objective of this study is to determine hormone measurement summaries that best explain the incidence of hot flashes in midlife women.

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Contributors

Catheryne Chiang participated in the performing of the experiments, analysis and interpretation of the data, and the writing of the paper.

Lisa Gallicchio participated in the conception, design, and performing of the experiments and the writing of the paper.

Howard Zacur participated in the conception, design, and performing of the experiments and the writing of the paper.

Susan Miller participated in the conception, design, and performing of the experiments and the writing of the paper.

Jodi A. Flaws participated in the conception, design, and performing of the experiments, contributed data, and participated in the writing of the paper.

Rebecca L. Smith participated in the conception, design, and performing of the experiments, the analysis and interpretation of the data, and the writing of the paper. All authors saw and approved the final version.</author_notes>

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

Written informed consent was obtained for all participants and the institutional review boards of the University of Illinois and Johns Hopkins University approved all protocols.

Provenance and peer review

This article has undergone peer review.

Research data (data sharing and collaboration)

Due to the personal nature of the questions asked in this study, raw data may be shared only upon IRB approval.

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Study Design: In a cohort study of 798 midlife women over 1 to 7 years, women provided 4 weekly blood samples annually and completed a survey detailing life history, ongoing behaviors, and menopausal symptoms. Estradiol, progesterone, and testosterone were measured in all serum samples. Annual summary variables of each hormone were median, mean, maximum, minimum, variance, and range. The association of these values with hot flashes was assessed using multivariable logistic regression and Bayesian network analysis, controlling for smoking history and menopausal status.

Main outcome measures: Hot flash incidence, severity, and frequency.

Results: For most outcomes, the best-fit model included progesterone variability; increased progesterone variance or range was correlated with decreased hot flash frequency (OR = 0.82, 95% CI = 0.74–0.91) and severity (OR = 0.82, 95% CI = 0.77–0.88). In the Bayesian network model, the maximum estradiol value was negatively correlated with many outcomes (OR for hot flashes = 0.68). Relationships between progesterone variability, maximum estradiol level, maximum progesterone level, and hot flashes indicate that the effects of progesterone variance on hot flash outcomes are likely mediated through progesterone's relationship with maximum estradiol level. Conclusions: Variability of progesterone, as opposed to mean values, should be used as an indicator of risk of hot flashes in midlife women.

Keywords

menopause; hot flashes; estradiol; progesterone

1. Introduction

Up to 87% of perimenopausal women experience vasomotor symptoms (hot flashes) [1]. These symptoms are associated with significant costs due to treatment (prescription, over-the-counter, alternative, and dietary), physician visits, laboratory testing, and loss of productivity; hormone therapy alone has been estimated to cost between \$357 and \$474 per patient per year [2]. The reasons that perimenopausal women experience hot flashes (HF) are unclear; no single factor has been consistently identified as playing a major role [3]. Many studies have shown the relationship between HFs and the mean levels of hormones, particularly estrogen withdrawal [1]. However, it is generally believed that decreases in estrogen levels are the primary reason for HFs [4] as the menopausal transition is a time of fluctuations in hormones and estrogen supplementation has been the most effective treatment [5]. While some studies have investigated how levels of hormones correlate with menopausal symptoms and the menopausal transition [3,6–8], limited information is available on the associations between hormonal fluctuations over a short period of time and HFs.

The objective of this study is to determine the hormone measures that best describe the HF experience in a cohort study of midlife women. These include estradiol, progesterone, and testosterone, measured as the mean, median, maximum, minimum, and variance.

2. Methods

2.1 Design and Participants

All participants gave written informed consent according to procedures approved by the University of Illinois and Johns Hopkins University Institutional Review Boards, which approved this research. The study design for the parent study is described in detail elsewhere [9]. Briefly, a cohort study of HFs among women 45–54 years of age was conducted starting in 2006 among residents of Baltimore and its surrounding counties. Women were recruited by mail and were included if they were in the target age range, had intact ovaries and uteri, and were pre-or perimenopausal. Exclusion criteria consisted of pregnancy, a history of cancer, exogenous female hormone or herbal/plant substance, and no menstrual periods within the past year. Participants made a baseline clinic visit, which included serum collection and completion of a detailed 26-page baseline survey. Participants then visited the clinic weekly for the next 3 weeks, providing serum samples at each visit. After the baseline year, participants returned annually to complete a questionnaire, repeating all previous questions about hot flashes and smoking, and providing the 4 weekly serum samples as before. Blood samples were stored until measurement of hormone levels as described below.

The questionnaire asked if women had experienced HFs in the last year, in the last 30 days, and the severity and frequency of the majority of their HFs at the time of the visit. With regards to severity, descriptions were: mild (sensation of heat without sweating), moderate (sensation of heat with sweating), or severe (sensation of heat with sweating that disrupts usual activity). For this analysis, severity was also dichotomized into “moderate or severe” versus “none or mild”. With regards to frequency, descriptions were: every hour, every 2–5 hours, every 6–11 hours, every 12–23 hours, 1–2 days per week, 3–4 days per week, 5–6 days per week, 2–3 days per month, 1 day per month, less than 1 day per month, or never. For this analysis, frequency was categorized into “daily”, “weekly”, “monthly”, and “never” and dichotomized into “daily or weekly” versus “monthly or less”. Although self-report of HFs was not validated in this study against an objective measurement, self-report of HFs has been accepted as a valid measure by both the National Institute on Aging and the FDA [10,11]. The questionnaire also asked women to report if they currently smoked, formerly smoked, or never smoked, as well as their age. Menopause status was defined as follows: premenopausal women were those who experienced their last menstrual period within the past 3 months and reported 11 or more periods within the past year; perimenopausal women were those who experienced 1) their last menstrual period within the past year, but not within the past 3 months, or 2) their last menstrual period within the past 3 months and experienced 10 or fewer periods within the past year; postmenopausal women were those women who had not experienced a menstrual period within the past year.

2.2 Hormone Variability

Serum samples extracted from the collected blood samples were used to measure estradiol, testosterone, and progesterone levels in each sample using commercially available, previously validated enzyme-linked immunosorbent assay (ELISA) kits (DRG, Springfield, New Jersey, USA) [12–15]. The minimum detection limits and intra-assay coefficients of variation were as follows: estradiol 9.714 pg/ml; testosterone 0.083 ng/ml; and progesterone

0.045 ng/ml. The average inter-assay coefficient of variation for all assays was less than 5%. In the case of values lower than the detection limits for the assay, we used the limit of detection as the hormone value; of samples used for this analysis, 11/560 progesterone values were below the limit of detection, whereas no estradiol or testosterone levels were below the limit of detection. Each sample was measured in duplicate within the same assay. All estradiol values greater than 500 pg/ml (n=22), progesterone values greater than 40 ng/ml (n=1), and testosterone values greater than 10 ng/ml (n=7) were removed from the data set because they were extreme outliers and we could not account for the high values (i.e., they were not due to the presence of ovarian cysts). The selected hormones were measured because they are all primarily produced by the ovary and are markers of normal ovarian function and have feedback roles in the hypothalamic-pituitary-gonadal axis [16,17]. Additionally, estradiol and progesterone decline with the onset of menopause and can subsequently be used as markers of the menopausal transition in the study subjects [16]. Across each woman in each year, the following summary variables were calculated for each hormone: mean, median, maximum, minimum, range, and variance. All values were $\log_{10}$ transformed for analyses.

2.3 Univariable Analysis

The relationship between each summary value (mean, median, maximum, minimum, range, and variance) of each hormone (estradiol, progesterone, and testosterone) and each hot flash measurement was assessed using logistic regression (any in the last year, moderate/severe, weekly/daily, and in the last 30 days) or ordinal logistic regression (severity and frequency), with random effects to account for the year of the study and the individual.

2.4 Multivariable Analysis

The best fit multivariable model for each outcome was fit using receiver-operator characteristic (ROC)-based forward model selection, controlling for menopausal status and with and without the potential confounder of smoking history included in the model. Briefly, the model was fit with each of the potential variables added separately, and the area under the curve (AUC) of the ROC for each model was calculated. The model with the highest AUC was selected for that round, and the process was repeated additively until no additions produced a higher AUC than the previous round. A subgroup analysis was also conducted with only women categorized as postmenopausal, to determine the relationship among those without hormone cycling.

2.5 Bayesian Network Analysis

Using data from the baseline visit, a Bayesian Network was fit for the variable nodes of hot flashes in the last year; hot flashes in the last 30 days; moderate/severe hot flashes; weekly/daily hot flashes; mean, maximum, minimum, and variance of all hormones; age; and smoking status. Smoking status was divided into two variables: ever vs. never smoked, and current smokers vs. not currently smoking. Menopausal status was divided into premenopausal vs. perimenopausal; there were no postmenopausal women at baseline. Hormone summary levels were not allowed to be parents for smoking status, and HF outcomes were not allowed to be parents for hormone summary values. The number of parents for any variable was limited to 3. HFs in the last 30 days was forced to be a child to

hot flashes in the last year. Best-fit models were plotted to include all parental nodes to the outcomes. Vectors were plotted with the coefficient of the statistical relationship between the parent and child node. For categorical variables, these relationships are based on a logistic regression model. For continuous variables, these relationships are based on a linear regression model.

2.6 Model Fitting

Logistic regression models were fit using the *lme4* package [18] in R 3.4.2 [19]. Ordinal logistic regression models were fit using the *multgee* package [20] in R 3.4.2 [19]. The AUC of the ROC curve for the model predictions was calculated using the *pROC* package [21] for logistic regression models and the *HandTill2001* package [22] for ordinal logistic regression models. Bayesian Network models were fit using the *abn* package [23] in R version 3.4.2 [19].

3. Results

3.1 Study Participants

Women were only included from the first 4 years after enrollment. There were 763 women providing information for the analysis, with 189 having only 1 year, 114 having 2 years, 103 having 3 years, and 357 having all 4 years of data. The number of samples available to analyze for each of the hormones measured (estradiol, progesterone, and testosterone) are shown in Table 1a, and the population characteristics are shown in Table 1b.

3.2 Hormone Variability

The distribution of the summary variables relative to HF experience is shown in Figure 1. Individuals providing only one sample in a year were not included in the variance and range variables. The correlation matrix of the hormones is shown in Supplemental Figure 1.

3.3 Univariable Analysis

All summary variables for estrogen and progesterone were significantly associated with all outcomes ($p < 0.001$ for all comparisons), with increases in any summary variable associated with decreases in the probability of any output (data not shown). The range of testosterone values was only significantly associated with the two outcomes measuring severity ($p = 0.029$ for the ordinal outcome and $p = 0.005$ for the dichotomous outcome, data not shown). All other summary variables for testosterone were significantly associated with all outcomes ($p < 0.05$), with increases in any summary variable associated with decreases in the probability of any output (data not shown).

3.4 Multivariable Analysis

The results of the multivariable logistic analysis are shown in Tables 2–5. Smoking status was correlated with several HF outcomes. Specifically, never smoking and former smoking was correlated with a decrease in HF frequency, HF severity, experiencing HFs in the last year, and experiencing HFs in the last 30 days when compared to current smokers (Tables 2–5). Menopausal status was correlated with all measured HF outcomes. Pre-menopausal

status was negatively correlated with all HF outcomes, whereas post-menopausal status was positively correlated with all HF outcomes when compared to peri-menopausal status (Tables 2–5). Progesterone variance was correlated with decreased frequency and severity of HF (Tables 4 and 5).

Among postmenopausal women only, smoking was significantly correlated with experiencing HFs in the last year and HF severity, with effects similar to the full analysis. No hormone measures were significantly associated with any outcomes among postmenopausal women, although the median progesterone level was included in the model for experiencing HF in the last year (OR: 0.07, $p = 0.14$), the minimum testosterone level was included in the model for HF severity (OR: 0.92, $p=0.81$), and the model for HF frequency included the variance of estradiol (OR: 0.88, $p=0.17$), median testosterone (OR: 129.9, $p=0.21$), mean testosterone (OR: 0.01, $p=0.22$), and mean estradiol (OR: 0.99, $p=0.99$).

3.5 Bayesian Network Analysis

The result of the Bayesian Network analysis are shown in Figure 2. Variables that were not correlated with HF outcomes are not shown. Smoking was associated with probability of any HFs. Higher probability of any HFs was associated with lower maximum estradiol as well as menopausal status. The relationship between maximum progesterone and frequency of HFs was mediated through maximum estradiol levels, which were negatively associated with experiencing any HFs. Menopausal status was associated with hormonal outcomes as well as HF outcomes. The relationship menopausal status shares with HF outcomes was partially mediated by the negative association between maximum estradiol and probability of any HFs, while the relationship between menopausal status and the frequency of hot flashes was partially mediated by the negative association between maximum progesterone and the frequency of HFs.

4. Discussion

Our results found that progesterone variance was the most important hormonal measurement in terms of HF experience. Specifically, when controlling for menopausal status and smoking history, an increase in progesterone variance was associated with a decrease in frequency and severity of HFs. Using a multivariate machine learning approach, most HF outcomes were ultimately explained by the variance of progesterone, history of smoking, and menopausal status. Previous analyses of these data [24–27] and similar studies [3,12] have found associations between mean hormone levels and HF experience, but this study shows that the predictive ability of the models was maximized by using summary values more related to variability. It is known that estradiol variability is associated with depressive symptoms during the menopausal transition [28], but to our knowledge, this is the first analysis to show that the variability of progesterone is highly associated with HF symptoms. Progesterone has long been suspected to play a role in vasomotor symptoms, as progesterone receptors have been found in the hypothalamus and progesterone can alter gene expression within serotonin neurons [29]. Progesterone therapy is known to reduce vasomotor symptoms, and may improve the efficacy of low-dose estrogen therapy [5]. It is possible that

the associations we found between progesterone variability and decreased HF symptoms may be explained by the variance of progesterone throughout the menstrual cycle, indicating that these women with increased progesterone variability and, subsequently, decreased HF symptoms, may still be experiencing full menstrual cycles. The menstrual cycle is commonly divided into the follicular phase and the luteal phase [30]. In the follicular phase, follicles within the ovary begin to mature and a dominant follicle arises and stimulates the preovulatory luteinizing hormone (LH) surge, leading to ovulation and release of the oocyte from the follicle. Following ovulation, the corpus luteum (CL) is formed and the luteal phase begins. During this time, the CL produces large amounts of progesterone as well as estradiol. Progesterone and estradiol production both decline with the degeneration of the CL and the beginning of the new follicular phase, whereas estradiol will slowly begin to rise again with the growth of the dominant follicle. Because estradiol is present in both the follicular and the luteal phase in addition to the preovulatory estradiol spike being relatively brief, it is perhaps not surprising that maximum estradiol, as opposed to variance of estradiol, was associated with reduced HF symptoms in women who donated samples once a week for four weeks. In contrast, progesterone is produced in very low quantities in the follicular phase and very high quantities in the luteal phase, possibly leading to samples over the course of four weeks from an actively cycling woman (i.e., a woman not likely to be experiencing more severe HF symptoms) having more variable readings of progesterone. Thus, it is possible that variance of progesterone in addition to measurements of maximum estradiol and maximum progesterone can be used as an indicator of cycling status in midlife women and, therefore, a predictor of their risk of experiencing HFs. This is somewhat confirmed by the results of the subgroup analysis of postmenopausal women, which did not include progesterone variability in any model.

Many possible summary variables were associated with many possible HF-related outcomes in this study, which raised a potential for false association due to multiple testing. However, some models found an association with the variance of progesterone, which indicates that this measure is associated with the experience of HFs. In addition, the Bayesian Network approach, which is designed to allow the discovery of complex relationships with many factors, found that the connection between hormone values and HF experience was limited to a few summary variables: progesterone variance, maximum progesterone, and maximum estradiol.

Many of the hormone variables were highly correlated, which could lead to multicollinearity in model fitting. However, the forward-stepping model selection procedure allowed for addition of the variables most improving the predictive ability of the model; highly correlated variables would not be likely to improve the model prediction.

This study was also limited by the self-reporting of HF symptoms, which can be subjective. However, self-reporting corresponds to clinical relevance, as women who report symptoms are more likely to seek treatment. In addition, only smoking and menopausal status were included as potential confounders; other factors, such as BMI, have been suggested to impact HF outcomes. However, our previous work has found that smoking and menopausal status are the most consistent factors associated with HF experience [26].

The findings of this study indicate that one hormone measurement, or summarizing multiple hormone measurements by averaging them, is not the best way to predict HF experience. Given that there is evidence that hormone fluctuations are also important predictors of depressive symptoms during menopause [31], measurement of hormones in studies of perimenopausal women should be longitudinal in nature. In conclusion, this study demonstrates a relationship between progesterone variability and HF experience. The underlying causes of perimenopausal HFs remain elusive, and future studies should be conducted to further elucidate the complex relationships shared between hormonal milieu and HF experience. Future studies investigating these relationships should consider more than measurements of mean hormone levels and that hormone levels across time may prove to be an important factor in the investigation thereof.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Progesterone variance was an important predictor of hot flash experience.
- Increased progesterone variance was associated with decreased hot flash frequency.
- Increased progesterone variance was associated with decreased hot flash severity.

Figure 1:
Distribution of parameter values. Values are divided among women experiencing (blue, right) or not (purple, left) hot flashes in the last year. Values are shown as log-transformed. Bars indicate the median value of each distribution.

Figure 2:

Bayesian Network for hot flash experience. Black/solid lines are outcomes, red/dotted lines are progesterone, blue/dashed lines are estradiol, and purple/dash-dot lines are the potential confounders smoking history and menopausal status. Lines leading into progesterone and estradiol boxes are coefficients from linear regression models. Lines leading into outcome boxes are coefficients from logistic regression models.

Table 1a:

Number of woman-year combinations providing multiple samples analyzed for hormone levels

	Number of samples provided			
	1	2	3	4
Estrogen	38	242	336	1579
Progesterone	37	241	324	1592
Testosterone	37	241	325	1592

Table 1b:

Description of women in data analyzed

Variable	Level	No Hot Flashes		Hot Flashes	
		number	%	number	%
Frequency	none	1011	0.49	0	0
	monthly	0	0	387	0.19
	weekly	0	0	294	0.14
	daily	0	0	317	0.15
Severity	none	1011	0.49	0	0
	mild	0	0	387	0.19
	moderate	0	0	587	0.28
	severe	0	0	82	0.04
Hot Flashes in the last 30 days	no	1011	0.49	152	0.07
	yes	0	0	894	0.43
Smoking status	Current	67	0.03	136	0.07
	Former	338	0.16	425	0.2
	Never	606	0.29	507	0.24
Menopause status	peri	257	0.12	559	0.27
	post	50	0.02	225	0.11
	pre	704	0.34	284	0.14
		mean	s.d.	mean	s.d.
Estrogen	median	1.71	0.3	1.52	0.33
	mean	1.76	0.29	1.55	0.33
	max	1.94	0.34	1.69	0.39
	min	1.47	0.32	1.33	0.31
	variance	2.65	1.2	1.8	1.63
	range	1.53	0.96	1.04	1.26
Progesterone	median	−0.13	0.62	−0.58	0.6
	mean	0.09	0.63	−0.45	0.67
	max	0.41	0.74	−0.24	0.8
	min	−0.77	0.45	−0.94	0.4
	variance	0.08	1.59	−1.31	1.71
	range	0.21	1.04	−0.6	1.15
Testosterone	median	−0.49	0.3	−0.52	0.28
	mean	−0.48	0.3	−0.51	0.28
	max	−0.39	0.29	−0.43	0.27
	min	−0.59	0.33	−0.62	0.3
	variance	−2.29	0.54	−2.34	0.51
	range	−1.02	0.59	−1.06	0.58

Table 2:

Multivariable results for hot flashes in the last year. Area under the ROC curve = 0.802

	OR	Estimate	Std. Error	z value	Pr(> z)
Former smoker	0.68 (0.47, 0.98)	−0.39	0.19	−2.09	3.66E-02
Never smoker	0.44 (0.31, 0.62)	−0.83	0.18	−4.58	4.76E-06
Post-menopause	2.12 (1.49, 3.03)	0.75	0.18	4.15	3.29E-05
Pre-menopause	0.18 (0.14, 0.22)	−1.71	0.11	−15.30	7.09E-53

Table 3:

Multivariable results for hot flashes in the last 30 days. Area under the ROC curve = 0.797

	OR	Estimate	Std. Error	z value	Pr(> z)
Former smoker	0.57 (0.4, 0.82)	−0.56	0.18	−3.06	0.0022
Never smoker	0.39 (0.27, 0.55)	−0.94	0.18	−5.29	1.25E-07
Post-menopause	2.61 (1.86, 3.66)	0.96	0.17	5.58	2.42E-08
Pre-menopause	0.21 (0.16, 0.26)	−1.58	0.11	−14.14	2.21E-45

Table 4:

Multivariable results for hot flash severity. Area under the ROC curve = 0.505

	OR	Estimate	Std. Error	z value	Pr(> z)
Former smoker	0.6 (0.448 – 0.814)	0.504	0.152	3.316	0.001
Never smoker	0.37 (0.275 – 0.489)	1.002	0.146	6.85	<0.001
Post-menopause	1.28 (1.001 – 1.645)	−0.249	0.127	−1.965	0.049
Pre-menopause	0.31 (0.244 – 0.386)	1.18	0.116	10.132	<0.001
Variance of Progesterone	0.82 (0.772 – 0.877)	0.195	0.032	6.005	<0.001

Table 5:

Multivariable results for hot flash frequency. Area under the ROC curve = 0.508

	OR	Estimate	Std. Error	z value	Pr(> z)
Former smoker	0.57 (0.42, 0.77)	0.57	0.16	3.58	<0.001
Never smoker	0.44 (0.32, 0.59)	0.83	0.16	5.27	<0.001
Post-menopause	1.48 (1.13, 1.95)	−0.39	0.14	−2.8	0.01
Pre-menopause	0.26 (0.21, 0.33)	1.35	0.12	11.7	<0.001
Variance of Progesterone	0.82 (0.74, 0.91)	0.2	0.05	3.89	<0.001
Minimum Progesterone	0.81 (0.58, 1.12)	0.21	0.17	1.29	0.2
Median Progesterone	0.9 (0.65, 1.26)	0.1	0.17	0.6	0.55