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ORIGINAL ARTICLE

Daily salivary cortisol patterns in midlife women with hot flashes

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Summary

Objective Diurnal salivary cortisol patterns in healthy adults are well established but have not been studied in midlife women with hot flashes. We hypothesized that frequent hot flashes are associated with aberrant cortisol patterns similar to sleep-deficient individuals.

Design Cross-sectional.

Participants A total of 306 women, ages 40–62, randomized to a behavioural intervention for hot flashes.

Measurements Baseline comparisons of cortisol geometric means (nmol/l) from four daily time points averaged over two consecutive days plus other calculated cortisol measures were made between groups defined by baseline: (i) mean daily hot flash frequency tertile (≤5·5, $N = 103$; >5·5–8·8, $N = 103$; >8·8, $N = 100$) and (ii) selected characteristics. Repeated-measures linear regression models of log-transformed cortisol evaluated group differences, adjusting for covariates.

Results Women were 67% White and 24% African American, with 7·6 (SD 3·9) hot flashes per day. Salivary cortisol geometric means (nmol/l) among all women were as follows: 75·0 (SD 44·8) total, 8·6 (SD 5·6) wake, 10·0 (SD 7·5) wake +30 min, 3·7 (SD 3·3) early afternoon and 1·6 (SD 1·8) bedtime. Wake + 30-minute values showed an 18% median rise from wake values (interquartile range −24 to 96%), and means varied by hot flash frequency tertile, from lowest to highest: 11·4(SD 7·3), 10·3 (SD 6·5) and 8·6 (SD 7·8), respectively, $P = 0·003$. Beside the early afternoon value ($P = 0·02$), cortisol values did not vary by hot flash frequency.

Conclusion Taken together, these findings suggest that high frequency of moderate-to-severe hot flashes may be associated with subtle abnormalities in cortisol concentrations – a pattern consistent with chronic sleep disturbance.

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Introduction

The hypothalamic–pituitary–adrenal (HPA) axis plays a primary role in the body's acute reactions to stress by balancing hormone releases from the adrenalin-producing adrenal medulla and from the corticosteroid-producing adrenal cortex. Acute stress is associated with an abrupt physiologic rise in cortisol.[1,2] Typical daily cortisol concentrations in healthy adults rise abruptly within 30 min of awakening (cortisol awakening response) and diminish throughout the day with lowest values in late evening.[3,4] Blunted cortisol responses (i.e. diminished awakening response or a lower diurnal variation) as well as lower daily overall concentrations are more apt to reflect chronic illnesses or stressors[1,3–5] and vary by race/ethnicity.[6–8] Late evening cortisol may be increased with chronic stress,[1,9] insomnia[10] and sleep disturbances.[11]

Daily cortisol patterns in midlife women bothered by hot flashes are understudied, but in theory, patterns may vary from normal healthy adults for three reasons. First, hot flashes have been associated with stress and anxiety.[12–14] Second, oestrogen may affect cortisol secretion.[15,16] Increased oestrogen variability has been associated with hot flashes (16), and it follows that women with more hot flashes could have aberrant daily cortisol patterns. Third, the majority of women with hot flashes report poor sleep.[17] In addition, insomnia and sleep disturbances[11] are associated with abnormal cortisol patterns.

Cortisol can be measured via the urine, serum or saliva, but salivary cortisol measurement has become the gold standard for ambulatory studies of stress and HPA axis regulation.[2] Measurement of ambulatory salivary free cortisol concentrations allows for frequent data collection in a natural setting with minimal participant burden and correlates with serum cortisol concentrations.[18] We hypothesized that women with more frequent moderate-to-severe hot flashes have different daily cortisol patterns as compared to women with fewer hot flashes. Secondly, we

hypothesized that women with more stress and anxiety would have more hot flashes and that stress, anxiety and higher hot flash frequency would strongly correlate with abnormal cortisol patterns. Our goal was to describe daily salivary free cortisol values and patterns in midlife women with hot flashes, to evaluate potential differences in cortisol by hot flash frequency, and to assess factors that might be associated with cortisol concentrations in midlife women with hot flashes.

Materials and methods

This study included 306 participants who provided salivary cortisol samples at baseline in a multicentre clinical trial conducted by the Menopausal Strategies: Finding Lasting Answers to Symptoms and Health (MsFlash) network. Details about the network are published.[19,20] This study was a 3 by 2 factorial, randomized, controlled trial conducted at 3 sites (Indianapolis, Oakland and Seattle), with the main results described elsewhere.[21–23] Eligible women were randomized to 12 weeks of yoga, exercise or usual activity, and simultaneously randomized to 1·8 g/day of omega-3 fish oil capsules (EPA 1275 mg, DHA 300 mg) or placebo capsules. Participants were recruited from February 2011 through January 2012, primarily by mass mailing to age-eligible women using purchased lists and health-plan enrolment files.

Women were ages 40–62 years, in the menopausal transition or postmenopausal or had a hysterectomy with FSH >20 mIU/ml and oestradiol ≤50 pg/ml, and in general good health. The term 'hot flash' is used to represent daytime and night-time hot flashes or night sweats. The hot flash eligibility criteria were as follows: ≥14 hot flashes per week recorded on daily hot flash diaries for 3 weeks, hot flashes rated as bothersome or severe on 4 or more occasions per week, and the hot flash frequency in week 3 did not decrease >50% from the average weekly levels in weeks 1 and 2. Exclusion criteria included as follows: body mass index (BMI) >37 kg/m^2; use of hormonal contraceptives or hormones in the past month; use of prescription or over-the-counter treatments for hot flashes/night sweats in the past month; unstable medical conditions; current user of one of the study interventions or a related activity (i.e. yoga, tai chi, qi gong, or meditation, regular exercise, omega-3 fatty acid supplements, frequent consumption of fish); contraindications to exercise or yoga (e.g. physical limitations), or omega-3 (e.g. allergy to soy or fish); or a major depressive episode in the past 3 months.

Salivary cortisol was collected for 2 consecutive days prior to randomization during the 2 weeks of baseline hot flash data collection, using the Salivette swab (Starstedt AG & Co, Lumbrecht, Germany) at 4 time points on each day: on awakening (wake), 30 min later (wake + 30), early afternoon and bedtime. The swab was refrigerated and placed in a home freezer within 4 h of collection. Specimens were brought in freezer bags to the study appointment within 1–3 weeks of collection and stored at −70 degrees Celsius until assayed. Participants were given written and verbal instructions not to brush their teeth but to rinse their mouth with water 10–15 min before collection. They were instructed to write the time and day on the collection form and to chew the Salivette swab for 45 s. Participants recorded the timing and presence of alcohol, caffeine, nicotine, intense physical activity and eating within 2 h of specimen collection.

Salivary free cortisol concentration (nmol/l) was assayed at the University of Washington, School of Nursing Laboratory, using a high-sensitivity cortisol enzyme immunoassay kit (Salimetrics, State College, PA, USA). The intra-assay coefficient of variation (CV) for means 0·16–2·07 (standard deviation [SD] 0·01–0·08) is 3–4%; interassay CV for means 0·43–1·99 (SD 0·01–0·05) is 3–4%.

Statistical analysis

Prior to analysis, cortisol values at each time point were set to missing if they did not meet specimen collection timing requirements. Wake specimens were required to have been collected between 4 and 11AM and within 15 min of awakening, wake + 30 between 15 and 60 min from wake time, the 'early afternoon' specimen between 4 and 8 h from awakening and the 'bedtime' measurement between 12 and 20 h from wakening.[7] This removed 1, 1, 37 and 10 women from the analyses, from the respective time points. Median and interquartile ranges for wake + 30, early afternoon and bedtime, with wake as time 0 were as follows: 30 min (30–32), 5·4 h (4·7–6·1) and 14·2 h (13·2–15·0). In addition to timing requirements, results were also set to missing if a participant reported possible contamination of the sample due to alcohol, caffeine, or nicotine use, intense physical activity or food intake within 2 h of specimen collection. We calculated three additional HPA axis measures[24]: (i) estimated daytime cortisol exposure (area under the curve [AUC]), (ii) awakening cortisol response, and (iii) diurnal variation. As the AUC and diurnal variation outcomes were dependent on the initial wake time of the participant, both outcomes were scaled to 14 h between wake and bedtime hour measurement, the median time for the study sample.

Participants were grouped by tertiles of the mean daily hot flash frequency (≤5·5, >5·5–8·8 and >8·8), calculated from 2 weeks of baseline hot flash diaries. Tests for trends in baseline characteristics across hot flash categories were estimated via linear (continuous, ordinal) or logistic (dichotomous) regression models. A number of baseline factors were chosen *a priori* as covariates in models of cortisol by hot flash frequency, including the following: (i) factors associated with cortisol differences as reported in other populations such as age, race (African American *vs* White)[6,8] and BMI (kg/m^2)[25]; (ii) clinical characteristics associated with cortisol or menopause[10,11,26,27] as measured by validated scales: depression [Patient Health Questionnaire (PHQ-8, range 0–24)], anxiety [General Anxiety Disorder Questionnaire (GAD 7, range 0–21)], stress [Perceived Stress Score (PSS, range 0–40)], sleep quality [Pittsburgh Sleep Quality Index (PSQI, range 0–21)] and insomnia [Insomnia Sleep Index (ISI, range 0–28)][19]; (iii) menopausal factors that might affect cortisol concentrations including status (postmenopausal *vs* menopause transition), hot flash severity (1–3 scale) and duration of hot flashes; and (iv) other factors known or suspected to affect cortisol and hot flashes: marital status, full-time employment

and cardiovascular risk factors (systolic and diastolic blood pressure).

The distributions of cortisol values were skewed at the 4 time points; thus, values were log-transformed. Geometric means and standard deviations (SD) were presented for the 2-day mean cortisol measurements by hot flash tertile. To evaluate the associations of cortisol and hot flash frequency, data from both days of cortisol collection were included as repeated measures in linear regression models of log cortisol values at each time point as a function of hot flash tertile, day (1st or 2nd) and clinical centre.

We evaluated associations of the following: (i) daytime cortisol exposure (AUC), (ii) cortisol awakening response and (iii) cortisol diurnal variation (wake minus bedtime) with hot flash frequency via a series of repeated-measures linear models. These particular measures have been shown to be abnormal in other

Table 1. Baseline characteristics of all women and by mean daily hot flash frequency

	All women		Mean daily hot flash frequency					
	N = 306		≤5·5 *N* = 103		>5·5 – 8·8 *N* = 103		>8·8 *N* = 100	
Baseline characteristic	N	%	N	%	N	%	N	%
Age at screening (years), mean (SD)*	54·6	(3·6)	55·2	(3·6)	54·6	(3·7)	54·0	(3·4)
≥55	149	48·7	57	55·3	49	47·6	43	43·0
Race								
White	204	66·7	69	67·0	68	66·0	67	67·0
African American	72	23·5	21	20·4	27	26·2	24	24·0
Other/unknown	30	9·8	13	12·6	8	7·8	9	9·0
Postmenopausal	250	81·7	87	84·5	84	81·6	79	79·0
History of hot flashes (years)								
<8	206	67·3	73	70·9	68	66·0	65	65·0
≥8	95	31·0	28	27·2	34	33·0	33	33·0
Hot flash severity (1–3 scale)†								
<Moderate (2)	181	59·2	86	83·5	59	57·3	36	36·0
≥Moderate	125	40·8	17	16·5	44	42·7	64	64·0
Married/living as married	207	67·6	65	63·1	73	70·9	69	69·0
Education								
≤High school/GED	13	4·2	5	4·9	2	1·9	6	6·0
Some college	98	32·0	31	30·1	33	32·0	34	34·0
College graduate	194	63·4	67	65·0	67	65·0	60	60·0
Full-time employed	187	61·1	64	62·1	68	66·0	55	55·0
Current smoker	21	6·9	7	6·8	5	4·9	9	9·0
BMI (kg/m^2), mean (SD)	26·8	(4·4)	27·0	(4·7)	27·0	(4·4)	26·3	(4·1)
>30	73	23·9	26	25·2	28	27·2	19	19·0
Systolic BP, mean (SD)	117·3	(13·5)	115·7	(12·8)	117·4	(13·4)	119·1	(14·1)
Diastolic BP, mean (SD)*	72·7	(9·1)	71·2	(9·5)	72·7	(8·6)	74·1	(9·0)
PHQ-8 Depression								
<5	194	63·4	67	65·0	67	65·0	60	60·0
≥5 (at least mild)	109	35·6	33	32·0	36	35·0	40	40·0
GAD-7 Anxiety								
<5	225	73·5	70	68·0	80	77·7	75	75·0
≥5 (at least mild)	81	26·5	33	32·0	23	22·3	25	25·0
PSS Stress								
<13·7	144	47·1	46	44·7	57	55·3	41	41·0
≥13·7 (above mean)	155	50·7	54	52·4	46	44·7	55	55·0
ISI Insomnia								
<8 (none-to-mild)	77	25·2	32	31·1	25	24·3	20	20·0
8–<15 (moderate)	135	44·1	44	42·7	42	40·8	49	49·0
≥15 (severe)	92	30·1	27	26·2	35	34·0	30	30·0
PSQI Sleep								
<5	43	14·1	18	17·5	12	11·7	13	13·0
≥5 (at least mild)	257	84·0	84	81·6	91	88·3	82	82·0

*T-test *P*-value <0·05 for hot flash frequency in a linear model with baseline characteristic as a function of linear trend hot flash frequency.
†Chi-square *P*-value <0·05 for hot flash frequency in a logistic model with baseline characteristic as a function of linear trend hot flash frequency.

populations with chronic stress, illness and sleep disturbances.[1,5,9–11] Three models estimated the association of cortisol with hot flash frequency for each of these cortisol measures. The first model adjusted for day and clinical centre; the second model additionally adjusted for demographic characteristics and body measurements; and the third model further adjusted for baseline depression, stress and insomnia.

An additional adjusted model estimated the association of cortisol outcomes with hot flash frequency in the subset of women with moderate-to-severe insomnia (ISI ≥ 15), a factor known to affect cortisol awakening response.[10] Factors evaluated as potential effect modifiers for the association of cortisol measures and hot flash frequency were determined *a priori*: stress (>13·7), insomnia (<8, 8–<15, ≥15) and anxiety (>5). Selected baseline factors from the entire study population were evaluated for their association with 2 cortisol measures: awakening response (wake + 30 min minus wake) and the log-transformed bedtime value using repeated-measures models adjusted for day (1st or 2nd), clinical centre, demographic characteristics, body measurements, baseline depression, stress and insomnia.

All statistical analyses were conducted in SAS for Windows version 9.4 (SAS Institute Inc. Cary, NC, USA). *P* values <0·05 were considered statistically significant.

Results

There were 306 women included: the mean and median ages were both approximately 55 years, over 80% were postmenopausal (median 3 years from final menstrual period), two-thirds were White, and almost two-thirds were college graduates (Table 1). Women had on average 7·6 (SD 3·9) hot flashes per day and reported having experienced hot flashes for a median duration of 5 years. Higher baseline hot flash frequency was associated with statistically significant lower age, higher diastolic blood pressure and higher hot flash bother/severity. Otherwise, the baseline characteristics did not vary by hot flash frequency.

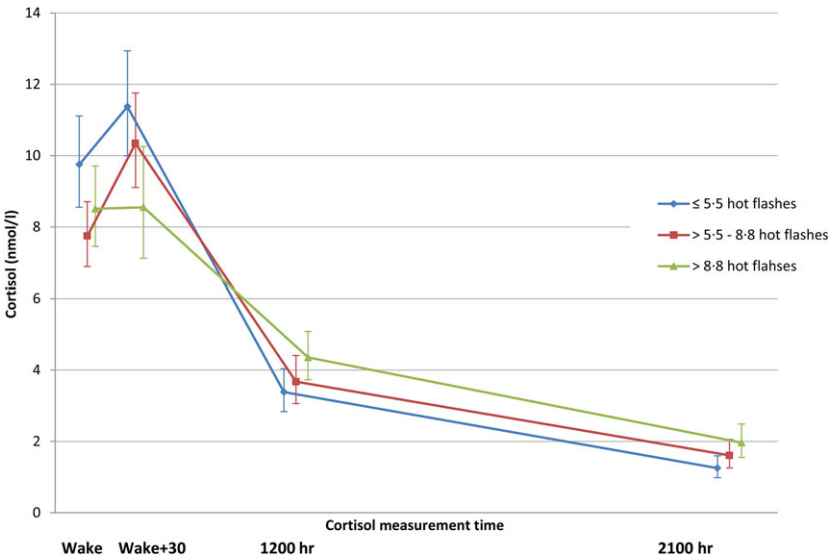

Fig. 1 Geometric mean salivary cortisol concentrations in 306 women over time, by mean daily hot flash frequency tertiles. *N* = 103, ≤5·5 mean daily hot flashes; *N* = 103, 5·5–8·8 mean daily hot flashes; and *N* = 100, >8·8 mean daily hot flashes.

Table 2. Cortisol geometric means and standard deviations by mean daily hot flash frequency

	All women		Mean daily hot flash frequency						
	N = 306		≤5·5 *N* = 103		>5·5 – 8·8 *N* = 103		>8·8 *N* = 100		
Cortisol measure (nmol/l)	Geo Mean	Geo SD	Geo Mean	Geo SD	Geo Mean	Geo SD	Geo Mean	Geo SD	*P*-value*
Wake	8·6	(5·6)	9·7	(6·5)	7·7	(4·6)	8·5	(5·6)	0·19
Wake + 30 min	10·0	(7·5)	11·4	(7·3)	10·3	(6·5)	8·6	(7·8)	0·003
Early afternoon	3·7	(3·3)	3·1	(3·2)	3·9	(2·9)	4·4	(3·7)	0·02
Bedtime	1·6	(1·8)	1·3	(1·5)	1·6	(1·8)	1·9	(2·1)	0·18

**P*-value from a repeated-measures linear model of log cortisol value as a function of linear hot flash tertile, adjusted for consecutive day (1st, 2nd) and clinical centre (Indianapolis, Oakland, Seattle).

Salivary free cortisol concentration geometric means (nmol/l) for all women were as follows: 75·0 (SD 44·8) total, 8·6 (SD 5·6) wake, 10·0 (SD 7·5) wake + 30 min, 3·7 (SD 3·3) early afternoon and 1·6 (SD 1·8) bedtime. The mean daily cortisol values for all women, averaged over the 2 days, followed a normal diurnal pattern (Fig. 1). The median per cent cortisol rise from awakening was 18% (interquartile range (IQR) −24 to 96%), but did not vary by hot flash frequency (≤5·5: 19% increase, IQR −18 to 91%; 5·5–8·8: 24% increase, IQR −20 to 107%; and >8·8: 10% increase, IQR −37 to 98%).

Cortisol values did not vary significantly by hot flash frequency, with the exception that the wake + 30-minute cortisol concentration was lowest ($P = 0{\cdot}003$) and early afternoon was highest among women with the greatest number of mean daily hot flashes (0·02) (Table 2). However, total daytime cortisol, awakening response, diurnal variation and bedtime cortisol did not vary by frequency of mean daily hot flashes in any of the adjusted models (Table 3). In a subsample of 92 women with ISI ≥ 15, a significant positive association with bedtime cortisol ($P = 0{\cdot}03$) was found (data not shown). Among this subsample, higher hot flash frequency tertiles were associated with decreased diurnal variation ($P = 0{\cdot}01$). The interactions between insomnia (ISI <8, 8–<15, ≥15), hot flashes and cortisol outcomes were not statistically significant, and there was no significant effect modification by stress or anxiety.

African American women had slightly lower morning cortisol values (both wake and wake + 30 min) and higher bedtime cortisol than White women, with a significantly lower awakening response ($P = 0{\cdot}05$) (Fig. 2, Table 4). Higher anxiety was significantly associated with decreased awakening response ($P = 0{\cdot}05$). None of the other selected baseline factors was significantly associated with cortisol awakening response or bedtime values.

Discussion

We evaluated daily salivary free cortisol patterns in midlife women with hot flashes at four time points on two consecutive days. Compared with reported normal ranges for salivary free cortisol in the general population,[26,27] women in our study appear to have had abnormally low or low range of normal values 8·6 (SD 5·6) wake, 10·0 (SD 7·5) wake +30 min, 3·7 (SD 3·3) early afternoon and 1·6 (SD 1·8) bedtime although direct comparisons cannot be made due to different assay methods. Cortisol values 30–40 min after awakening are typically 50–100% higher than awakening values in 75% of normal healthy adults.[3] However, in our sample, the median rise was only 18% higher than the wake value.

Our hypothesis that women with greater hot flash frequency would have a different pattern of cortisol compared to women with few hot flashes was supported. Higher hot flash frequency was significantly associated with lower mean cortisol values at the time point of wake + 30 min. Women with greater hot flashes also trended towards higher bedtime cortisol, and there was a trend towards a diminished diurnal variation among women with the greatest number of hot flashes; however, neither of these was statistically significant.

Table 3. Adjusted models of cortisol outcomes by mean daily hot flash frequency

		Mean daily hot flash frequency			
Cortisol outcome	Model	≤5·5 $N = 103$* Estimate (95% CI)†	>5·5 – 8·8 $N = 103$* Estimate (95% CI)†	>8·8 $N = 100$* Estimate (95% CI)†	*P*-value‡
Total daytime (AUC)§(log nmol/l)	1	0·00 (Ref)	−0·03 (−0·24, 0·17)	−0·01 (−0·21, 0·19)	0·94
	2	0·00 (Ref)	−0·06 (−0·26, 0·14)	0·01 (−0·21, 0·22)	0·99
	3	0·00 (Ref)	−0·04 (−0·24, 0·17)	−0·01 (−0·24, 0·23)	0·94
Awakening response (wake + 30 min minus wake) (nmol/l)	1	0·00 (Ref)	1·08 (−0·80, 2·97)	−1·02 (−2·92, 0·87)	0·30
	2	0·00 (Ref)	1·54 (−0·39, 3·47)	−0·59 (−2·58, 1·41)	0·58
	3	0·00 (Ref)	1·24 (−0·70, 3·17)	−1·08 (−3·08, 0·93)	0·30
Diurnal variation§(wake minus bedtime) (nmol/l)	1	0·00 (Ref)	−5·58 (−10·15, −1·00)	−4·23 (−8·72, 0·26)	0·07
	2	0·00 (Ref)	−6·10 (−11·12, −1·09)	−5·26 (−11·12, 0·61)	0·08
	3	0·00 (Ref)	−4·91 (−9·69, −0·12)	−4·57 (−10·88, 1·74)	0·15
Bedtime (log nmol/l)	1	0·00 (Ref)	0·18 (−0·16, 0·52)	0·22 (−0·10, 0·53)	0·18
	2	0·00 (Ref)	0·20 (−0·12, 0·53)	0·29 (−0·04, 0·61)	0·09
	3	0·00 (Ref)	0·18 (−0·15, 0·51)	0·29 (−0·05, 0·63)	0·10

*Following exclusions for time window, alcohol, caffeine, nicotine, intense physical activity and eating within 2 h of specimen collection models included the following numbers of women by hot flash tertile: total daytime 59, 55, 59; awakening response 94, 95, 93; diurnal variation 77, 72, 76; and bedtime 78, 72, 76.

†Estimates and 95% confidence intervals from a repeated-measures linear regression model represent the mean cortisol difference between groups with higher baseline hot flash frequency and the ≤5·5 group, after adjustment for other factors as described below.

‡*P*-values from a repeated-measures linear regression model of each outcome as a linear trend over hot flash frequency tertiles. Model 1: Adjusted for day, clinical centre; Model 2: Adjusted for factors in Model 1+ age, race, menopause status, marital status, full-time employment, BMI, systolic BP and diastolic BP; Model 3: Adjusted for factors in Models 1 and 2+ depression (PHQ-8), stress (PSS) and insomnia (ISI).

§Total AUC and diurnal variation are scaled to the median wake to bedtime measurement time, 14 h.

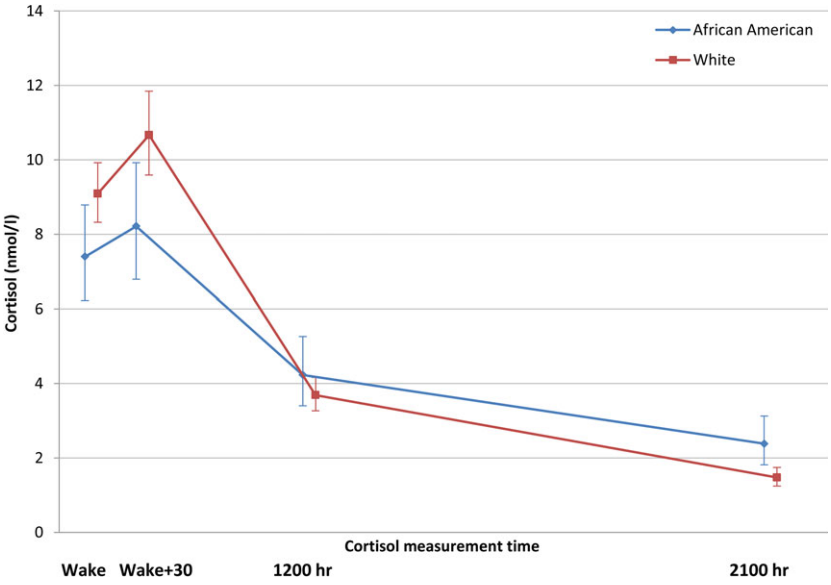

Fig. 2 Geometric mean salivary cortisol concentrations over time among 204 White and 72 African American women

Table 4. Adjusted models of cortisol outcomes by baseline factors other than hot flashes

		Cortisol outcome			
		Awakening response (nmol/l) $N = 282$		Bedtime (log nmol/l) $N = 226$	
Baseline characteristic	Subgroups	Estimate (95% CI)*	*P*-value*	Estimate (95% CI)*	*P*-value*
Race	African American *vs* White	−2·19 (−4·35, −0·03)	0·05	0·28 (−0·07, 0·63)	0·12
Menopause status	Post- *vs* Perimenopausal	1·24 (−0·64, 3·12)	0·20	−0·26 (−0·59, 0·07)	0·13
History of hot flashes	≥8 *vs* <8 years	0·25 (−1·82, 2·33)	0·81	0·29 (−0·03, 0·61)	0·08
Mean hot flash severity	>Moderate *vs* ≤Moderate	0·14 (−1·76, 2·03)	0·89	−0·02 (−0·36, 0·32)	0·91
BMI (kg/m^2)	≥30 *vs* <30	0·47 (−1·55, 2·50)	0·65	−0·26 (−0·60, 0·09)	0·15
PHQ-8 Depression	(≥mild) ≥5 *vs* <5	0·33 (−1·75, 2·42)	0·75	0·19 (−0·13, 0·50)	0·25
GAD-7 Anxiety†	(≥mild) ≥5 *vs* <5	−2·17 (−4·28, −0·07)	0·05	0·32 (−0·01, 0·64)	0·06
PSS Stress	(≥mean) ≥13·7 *vs* <13·7	−0·82 (−2·45, 0·81)	0·32	−0·11 (−0·39, 0·18)	0·46
ISI Insomnia‡	(moderate) 8–<15 *vs* <8	1·95 (0·05, 3·86)	0·59	0·11 (−0·27, 0·49)	0·54
	(severe) ≥15 *vs* <8	0·79 (−1·51, 3·09)		0·13 (−0·28, 0·54)	
PSQI Sleep Quality‡	(≥mild) ≥5 *vs* <5	−0·76 (−2·91, 1·39)	0·49	0·08 (−0·30, 0·47)	0·67

*Estimates, 95% confidence intervals, and *P*-values from a repeated-measures linear regression model with the outcome of interest as a function of the individual baseline characteristic of interest, adjusted for day, clinical centre, hot flash frequency, age, race, menopause status, marital status, full-time employment, BMI, systolic BP, diastolic BP, depression (PHQ-8), stress (PSS) and insomnia (ISI).
†GAD-7 Anxiety model not adjusted for PHQ-8 depression because of high correlation between anxiety and depression.
‡For *P*-value 3-level ISI was treated as a linear variable.
‡PSQI sleep model not adjusted for ISI insomnia because of high correlation between sleep quality and insomnia.

Researchers have suggested that the cortisol awakening response reflects phasic physiologic processes specific to the sleep–wake transition.[5] Given that the majority of women in the study reported poor sleep quality, it is possible that their poor sleep contributed to the decreased wake + 30-minute free cortisol concentrations associated with increased hot flashes; however, this did not translate into a lower awakening response in our study population. Among women with moderate-to-severe insomnia, both the bedtime cortisol value and diurnal rhythm were associated with the highest tertile of mean daily hot flash frequency. We are not aware of other studies that evaluated sleep, hot flashes and cortisol. However, associations between poor sleep and cortisol were described in the large Whitehall II study, where both sleep duration and sleep disturbances were independently associated with a flatter diurnal slope in cortisol secretion. In the Whitehall II study, evening cortisol secretion was higher in participants who reported short sleep duration and high sleep disturbance.[11] Similarly, in a small laboratory-based study of six men and five women with chronic insomnia, the evening cortisol serum concentrations were high compared to 13 healthy controls without sleep problems.[10]

Our hypotheses that women with more stress and anxiety would have more hot flashes and that stress, anxiety and higher hot flashes would strongly correlate with cortisol patterns were not supported. However, we did find that among all women with hot flashes, those with increased anxiety trended towards lower awakening response and higher bedtime cortisol concentrations. One small study correlated self-reported hot flashes with stress, although this was not corroborated when hot flashes were measured objectively.[14] Notably psychological stress has been cited as one of the most common triggers of hot flashes. The small number of women in our study who reported high anxiety or high stress made it unlikely to find significant associations.

We are not aware of other reports of associations between daily salivary cortisol patterns and hot flashes in midlife women (search terms: cortisol, menopause, hot flashes, November 2015). Other investigators evaluated *overnight urinary* free cortisol, a measure of total cortisol production and not daily cortisol patterns.[2] However, the findings indicated that midlife women with more severe hot flashes had increased overnight urinary cortisol compared to women who reported fewer hot flashes.[28] Similarly, another study found increased overnight urinary cortisol in women with higher Greene Climacteric scores in the early postmenopausal period.[29] In contrast, a longitudinal study showed higher overnight urinary cortisol was associated with a decrease in the number of hot flashes that same day or on the following day.[30] These mixed results suggest that overnight urinary cortisol may not be a good measure of chronic menopausal symptom burden, but instead is a marker of acute hot flash frequency (i.e. hot flashes measured the same day as cortisol measurement).

We did not have sufficient numbers of women in our study to examine the association of hot flashes, cortisol and race. However, African American women in our study, irrespective of hot flashes, had bedtime free cortisol concentrations significantly higher than those of White women and a trend towards morning cortisol concentrations significantly lower than those of White women. These findings are consistent with previous reports from larger population-based studies, where cortisol levels differed significantly between African American and White women.[6–8]

Limitations of our study are important to consider. Our study was not designed to identify associations of acute cortisol fluctuations with acute onset of a hot flash. Although a previous laboratory-based study with indwelling catheters in postmenopausal women evaluated acute cortisol response related to hot flashes and showed that an acute cortisol rise followed a hot flash,[31] these studies have not been replicated. Another limitation is the lack of a comparison group of women without hot flashes, and subtle differences in cortisol values could have been missed due to sample size. The fact that our results do not confirm prior studies[10,11,25,32] may be related to greater homogeneity among our sample on a variety of health parameters as a result of strict inclusion and exclusion criteria in the MsFLASH02 trial. Lastly, our sample was limited by excluding women who did not take their salivary samples within the specified time ranges or who had potential contamination of their samples. Despite the diminished sample size following these exclusions, this exploratory analysis provides important information for the design and execution of future studies.

Strengths of this study include a rigorous protocol used for the collection of salivary samples at multiple time points over 2 consecutive days and the specific parameters used in the selection of samples used for statistical analysis. Salivary cortisol is an excellent measure of unbound cortisol, and it is unaffected by factors that affect serum cortisol binding globulin.[18,33] The study included rich covariate data to evaluate associations of numerous potential confounders.[24] The sample was community-based and relatively diverse in demographic characteristics, including African American women. Most importantly, the study establishes norms for circadian cortisol concentrations in midlife women with hot flashes.

Taken together, these findings suggest that a greater frequency of moderate-to-severe hot flashes in midlife women may be associated with subtle abnormalities in free cortisol concentrations in a pattern consistent with chronic sleep disturbance. More than 80% of US women report hot flashes[34] and the majority rate them as moderate-to-severe, so the potential magnitude of the impact of our findings is not inconsequential. Individuals with chronic sleep disturbance have greater health risks[35] – the long-term health consequence of chronic frequent hot flashes and aberrant cortisol patterns warrants further study.

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