

ORIGINAL STUDY

Association of hot flushes with ghrelin and adipokines in early versus late postmenopausal women

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Abstract

Objective: Vasomotor flushing (hot flushes) is a common menopausal symptom experienced by most women going through the menopausal transition; flushing continues for a variable period in postmenopause. Primarily due to lack of ovarian estrogen, other biomarkers of hot flushes have not been clearly identified. We examined the relationship of hot flushes with ghrelin and adipokines.

Methods: Baseline data from two clinical trials, the Women's Isoflavone Soy Health (WISH) trial and Early versus Late Intervention Trial of Estrogen (ELITE), were used in this post hoc cross-sectional study. Both WISH and ELITE had similar study designs, inclusion criteria, and data collection processes. Study participants were healthy postmenopausal women not taking estrogen-based hormone therapy, free of cardiovascular disease, or any other chronic diseases. Both trials used the same hot flush diary in which participants recorded the number of daily hot flushes by severity over a month on average. Serum concentrations of ghrelin, leptin, adiponectin, and resistin were assessed in stored fasting blood samples using highly specific radioimmunoassay. In this analysis, self-reported flushing experience was tested for an association with leptin, adiponectin, resistin, and ghrelin concentrations using logistic regression and mean comparisons.

Results: A total of 898 postmenopausal women from the ELITE and WISH trials contributed to this analysis. Mean (SD) age was 60.4 (7.0) years, body mass index (BMI) 27 (5.3) kg/m², 67% were white, and 47% were within 10 years of menopause. Reported flushing was significantly associated with younger age, lower education, lower BMI, being married, and more recent menopause. Adjusted for these factors other than BMI, women in the highest quartile of ghrelin had significantly greater likelihood of experiencing hot flushes (OR [95% CI] = 1.84 [1.21-2.85]) compared to women in the lowest quartile. The association was more pronounced among overweight or obese women (OR [95% CI] = 2.36 [1.28-4.35]) compared to those with normal BMI (1.24 [0.54, 2.86]; interaction *P* value = 0.46). The association between ghrelin and hot flushes was similar among early (within 10 y) and late (over 10 y) postmenopausal women. Blood levels of adiponectin and resistin were not associated with hot flushes.

Conclusions: Higher concentrations of ghrelin were associated with greater likelihood of hot flushes in both early- and late-postmenopausal women. Leptin, adiponectin, and resistin levels were not associated with hot flushes in postmenopausal women.

Key Words: Adipokines – Ghrelin – Hot flushes – Menopause.

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Vasomotor flushing (hot flushes) is a common menopausal symptom experienced by most women during the menopausal transition.¹ Although the exact physiological mechanism of hot flushes is yet to be understood, accumulating evidence supports the “thermoregulatory hypothesis,” which includes a complex interplay between central and peripheral components.²⁻⁴ The thermoregulatory hypothesis posits that the sharp decline and eventual withdrawal of estradiol during the menopausal transition leads to narrowing of the thermoneutral zone in the central nervous system.⁵ As a result, a minor change in the core body temperature initiates a cascade neuroendocrine response in order to preserve the core temperature; vasomotor flushing is the ultimate outcome of this compensatory mechanism.

Research over the past two decades has identified the hypothalamic KNDy (Kisspeptin, neurokinin B, and dynorphin) neurons as the central modulator of hot flushes.⁶⁻⁸ According to the thermoregulatory hypothesis, body fat is a peripheral contributor to hot flushes, as adipose tissue can serve as an insulator and inhibit heat dissipation.⁹ Studies evaluated the role of adipose tissue-derived cytokine-like substances, collectively known as adipokines, as an additional mechanism that might explain the link between adiposity and flushing. Adjusted for age and time since last menstrual period, higher leptin and lower adiponectin levels were associated with increased hot flushes in midlife women,^{10,11} particularly among women early in the menopausal transition.¹¹ The majority of such research has originated from the Study of Women's Health Across the Nation (SWAN) and the Midlife Women's Health Study (MWHS)¹² of women going through the menopausal transition, suggesting cessation of ovarian estradiol as the primary cause of flushing. Notably, approximately 30% of women continue to experience flushing through late postmenopause.¹³⁻¹⁵ The association of adipokines with hot flushes has not been evaluated in older postmenopausal women who are likely to gain weight.

Although adipokines have been investigated in relation to flushing, ghrelin, the orexigenic peptide hormone released by the endocrine cells in the stomach, has not been evaluated in relation to hot flushes. Ghrelin stimulates growth hormone secretion, increases appetite, adiposity, and plays an important role in energy homeostasis.¹⁶ We previously reported associations of adipokines and ghrelin with sex steroid hormones and sex hormone-binding globulin in postmenopausal women.¹⁷ In that report, we observed an inverse association between estradiol and ghrelin; however, the association was not independent of body mass index (BMI). We also examined the association across BMI strata and found the inverse association between ghrelin and estradiol was statistically significant only among obese women. To our knowledge, the role of ghrelin in vasomotor flushing has not been evaluated. For the current investigation, we hypothesized that higher ghrelin levels would be associated with increased flushing in postmenopausal women. We also evaluated the relationship of adipokines with hot flushes in this group of postmenopausal women with a wide range of time since menopause and evaluated if adipokine associations differed across BMI categories, and between early (≤ 10 y) versus late (≥ 10 y) postmenopausal women.

MATERIALS AND METHODS

Study population

Baseline data from two completed clinical trials were included in this post hoc analysis. The Women's Isoflavone Soy Health (WISH) trial and Early versus Later Intervention Trial with Estradiol (ELITE) were randomized, double-blind, placebo-controlled trials conducted from April 2004 to March 2009 and from July 2005 to February 2013, respectively. Both trials were conducted at the University of Southern California Atherosclerosis Research Unit; primary results have been

reported.^{18,19} Briefly, 350 postmenopausal women in the WISH trial were randomly assigned to daily 25 g soy protein or daily total milk protein-matched placebo, whereas 643 postmenopausal women in the ELITE study were randomly assigned to oral 17 β -estradiol (1 mg daily) or matched placebo.¹⁹ Study design and data collection protocols were similar. Healthy postmenopausal women with no history of chronic illness including cardiovascular disease, diabetes, kidney or thyroid disease, or cancer in the past 5 years, and who had not taken exogenous hormone therapy in the past 12 months were eligible. Postmenopausal status was based on a serum level of total E₂ < 25 pg/mL and the absence of vaginal bleeding for at least six months (natural menopause) or bilateral oophorectomy (surgical menopause). Demographic information and overnight fasting blood samples were collected at the baseline visit; samples were stored at -80°C . Written informed consent was obtained from all participants; each trial was approved by the Institutional Review Board of the University of Southern California, and was registered on clinicaltrials.gov (WISH:NCT00118846, ELITE:NCT00114517).

Flushing outcome

Participants of WISH and ELITE studies completed identical hot flush diaries that included questions about frequency and intensity of hot flushes they experienced each day over the past week. A weekly composite flushing score was calculated from the self-reported monthly hot flush diary. In this analysis, we defined flushing experience as a binary indicator for none, or at least one hot flush of any intensity over the week reported. Based on reported intensity of hot flushes, women were categorized into three groups: (0) no hot flushes, (1) mild hot flushes only, or (2) mixture of mild, moderate, and severe hot flushes.

Independent variables

Adipokines (leptin, adiponectin, and resistin), and ghrelin were measured from stored fasting samples collected at the baseline visit and stored at -80°C . All assays were conducted in a blinded fashion in the Reproductive and Endocrine Laboratory at the University of Southern California. Leptin, adiponectin and ghrelin were measured in serum by highly specific radioimmunoassay using reagents obtained from Linco Research (St. Charles, MO). The leptin assay sensitivity is 0.5 ng/mL, and the interassay coefficients of variation (CVs) are 6.2%, 4.7%, and 3.6% at 4.9 ng/mL, 10.4 ng/mL, and 25.6 ng/mL, respectively. The assay sensitivity for adiponectin is 1 ng/mL, and the interassay CVs are 9.2%, 6.9%, and 9.2% at 1.5 ng/mL, 3.0 ng/mL, and 7.5 ng/mL, respectively. Total ghrelin was also quantified by a competitive radioimmunoassay. The assay sensitivity is 93 pg/mL, and the interassay CVs are 14.7%, 16.0%, and 16.7% at 1 ng/mL, 2 ng/mL, and 3 ng/mL, respectively. Resistin was measured by enzyme linked immunosorbent assay using reagents from Linco Research. The assay has a sensitivity of 0.16 ng/mL and the interassay CVs are 7.7% and 9.1% at 0.67 ng/mL and 3.73 ng/mL, respectively.

Statistical analysis

Demographic characteristics were compared between women experiencing hot flushes or no hot flushes using independent *t* test and chi-square test depending on the nature of the variable. Levels of adipokines and ghrelin were compared between women with or without flushing experience using independent *t* test. Linear regression models were used to compare the mean levels of adipokines and ghrelin adjusted for demographic factors that were significantly different between flushers versus nonflushers. Adipokines and ghrelin were categorized using quartiles of their distributions. Logistic regression was used to evaluate the associations of adipokines and ghrelin with the binary hot flush variable. Since BMI, an indicator of adipose tissue, is the primary source of adipokines, the multivariate models did not include BMI as a covariate. We evaluated the association of the following additional independent variables with flushing: age, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander, and other), years of education, BMI classification (kg/m²), waist-to-hip ratio, smoking status, and history of hormone therapy use for menopausal symptoms. Multinomial logistic regression models were used to evaluate the association of ghrelin and adipokines (trend across quartile categories) with hot flush intensity (none, mild, mixed intensity). Regression models were additionally stratified by BMI (normal [<25 kg/m²], overweight or obese [≥ 25 kg/m²]) and years-since-menopause (<10 y vs ≥ 10 y). Statistical tests for interaction were conducted by including a product term between each independent variable of interest and BMI category or time since menopause category.

Statistical analyses used SAS 9.2 software (SAS, Inc., Cary, NC); statistical testing was conducted at a 2-tailed 0.05 significance level.

RESULTS

A total of 898 postmenopausal women (331 WISH, 567 ELITE) contributed to this analysis; hot flush data were missing for 95 women. Age, education, marital status, and years-since-menopause statistically significantly differed in women who did versus did not report flushes (Table 1). Women experiencing hot flushes were significantly younger, with lower BMI, less education, and were more likely to be married, and to be within 10 years of menopause. Compared to women with no flushing experience, ghrelin level was

TABLE 1. Demographic characteristics by self-reported flushing

Characteristics	No flushing (n = 399)	Any flushing (n = 499)	<i>P</i> ^a
Age, y	62.5 (7.0)	58.6 (6.5)	<0.0001
Race			
White non-Hispanic	260 (65%)	348 (70%)	0.40
Black non-Hispanic	30 (8%)	37 (7%)	
Hispanic	63 (16%)	62 (12%)	
Asian/Pacific Islander	39 (10%)	45 (9%)	
Other	7 (1%)	7 (2%)	
Marital status			
Single/never married	37 (9%)	37 (7%)	0.0002
Married	196 (49%)	320 (64%)	
Other	166 (42%)	142 (29%)	
BMI, kg/m ²			
Normal <25	147 (37%)	212 (43%)	0.04
Overweight (25-29)	144 (36%)	176 (35%)	
Obese (≥ 30)	108 (27%)	111 (22%)	
Mean (SD)	27.4 (5.58)	26.7 (5.09)	0.04
Waist/Hip ratio	0.8 (0.06)	0.8 (0.06)	0.69
Education, y	15.9 (2.37)	16.2 (2.14)	0.02
Smoking			
No	233 (58%)	304 (61%)	0.43
Former	153 (38%)	185 (37%)	
Current	13 (3%)	10 (2%)	
Type of menopause			
Natural	362 (91%)	459 (92%)	0.67
Surgical	35 (8%)	40 (8%)	
Unknown	2 (1%)	0	
Years-since-menopause			
Early (<10 y)	152 (38%)	279 (56%)	<0.0001
Late (≥ 10 y)	218 (55%)	181 (36%)	
Unknown	29 (7%)	39 (8%)	
Mean (SD)	12.8 (8.2)	9.1 (7.4)	<0.0001
Any past use of hormone therapy			
Yes	281 (70%)	362 (73%)	0.48
No	118 (30%)	137 (27%)	

BMI, body mass index.

^aChi square test for categorical variables, *t* test for continuous variables.

significantly higher among women experiencing hot flushes adjusted for age, years-since-menopause, education, and marital status (*P* value for difference = 0.005) (Table 2). Leptin, adiponectin, and resistin levels were not significantly different between women with and without flushing experience.

Adjusted for these hot flush-associated factors other than BMI, women in the highest quartile of ghrelin were 1.84 (95% CI 1.21-2.85) times as likely to report flushing compared to women in the lowest quartile (Table 3). The prevalence of hot flushes increased with increasing ghrelin concentrations (*P* value for trend = 0.007). Although the interaction was

TABLE 2. Comparison of adipokines and ghrelin levels by flushing status

	Unadjusted			Adjusted ^a		
	No flushing	Any flushing	<i>P</i>	No flushing	Any flushing	<i>P</i>
	Mean (SEM)	Mean (SEM)		Mean (SEM)	Mean (SEM)	
Ghrelin, pg/mL	7.04 (0.02)	7.10 (0.02)	0.01	7.03 (0.02)	7.10 (0.02)	0.005
Leptin, ng/mL	2.76 (0.04)	2.67 (0.03)	0.08	2.75 (0.04)	2.69 (0.03)	0.22
Adiponectin, μ g/mL	2.58 (0.02)	2.57 (0.02)	0.82	2.57 (0.02)	2.58 (0.02)	0.82
Resistin, ng/mL	2.54 (0.03)	2.53 (0.02)	0.72	2.54 (0.03)	2.54 (0.02)	0.95

SEM, standard error of mean.

^aAdjusted for age, time since menopause, education, and marital status.

TABLE 3. Association between hot flushes and adipokines and ghrelin in postmenopausal women

	Stratified by BMI				
	Total sample	Normal < 25 kg/m ²		Overweight and obese ≥ 25 kg/m ²	
		OR ^a (95% CI)	OR ^a (95% CI)	OR ^a (95% CI)	Stratified by years-since-menopause
				<10 y	>10 y
	OR ^a (95% CI)	OR ^a (95% CI)	OR ^a (95% CI)	OR ^a (95% CI)	OR ^a (95% CI)
Ghrelin, pg/mL					
<929	Ref	Ref	Ref	Ref	Ref
930-1,187	1.14 (0.76-1.78)	0.96 (0.39-2.36)	1.14 (0.71-1.84)	1.17 (0.65-2.12)	1.11 (0.63-1.97)
1,188-1,530	1.16 (0.80-1.74)	0.97 (0.41-2.29)	1.10 (0.67-1.82)	1.24 (0.71-2.19)	1.02 (0.56-1.85)
>1,530	1.84 (1.21-2.85)	1.24 (0.54-2.86)	2.36 (1.28-4.35)	1.86 (1.03-3.36)	1.75 (0.97-3.19)
Trend <i>P</i> value	0.007	0.42	0.02	0.046	0.095
Leptin, ng/mL					
≤9.6	Ref	Ref	Ref	Ref	Ref
9.7-16	0.81 (0.53-1.22)	0.75 (0.44-1.26)	1.38 (0.59-3.23)	0.82 (0.47-1.44)	0.82 (0.45-1.48)
17-26.5	1.18 (0.78-1.78)	1.63 (0.75-3.53)	1.89 (0.83-4.27)	1.25 (0.69-2.27)	1.11 (0.62-1.97)
>26.5	0.69 (0.46-1.04)	0.15 (0.02-1.31)	1.26 (0.56-2.83)	0.65 (0.36-1.15)	0.75 (0.41-1.34)
Trend <i>P</i> value	0.27	0.68	0.98	0.33	0.35
Adiponectin, μg/mL					
≤9.6	Ref	Ref	Ref	Ref	Ref
9.7-13.28	0.94 (0.63-1.40)	1.11 (0.52-2.36)	0.84 (0.49-1.31)	0.93 (0.53-1.65)	0.90 (0.50-1.63)
13.29-18	0.89 (0.59-1.34)	0.99 (0.47-2.08)	0.77 (0.46-1.28)	0.73 (0.41-1.29)	1.05 (0.58-1.88)
>18	1.03 (0.68-1.56)	0.87 (0.42-1.78)	1.12 (0.65-1.94)	0.88 (0.48-1.60)	1.17 (0.66-2.09)
Trend <i>P</i> value	0.97	0.53	0.95	0.48	0.49
Resistin, ng/mL					
≤9.5	Ref	Ref	Ref	Ref	Ref
9.6-13	1.16 (0.73-1.85)	1.39 (0.74-2.61)	1.22 (0.71-2.07)	0.88 (0.50-1.56)	1.88 (1.06-3.36)
13.5-17.8	1.11 (0.75-1.66)	1.21 (0.64-2.28)	0.93 (0.55-1.59)	0.88 (0.48-1.59)	1.28 (0.73-2.27)
>17.8	0.90 (0.60-1.35)	1.02 (0.52-1.98)	0.85 (0.50-1.43)	0.73 (0.41-1.29)	1.18 (0.65-2.16)
Trend <i>P</i> value	0.46	0.98	0.36	0.29	0.88

BMI, body mass index.

^aAdjusted for age, education, marital status, and years-since-menopause.

not statistically significant ($P=0.46$), the association between ghrelin and hot flushes was more pronounced among the overweight or obese women (OR [95% CI] =2.36 [1.28-4.34]) compared to women with ideal BMI (OR [95% CI] = 1.24 [0.54-2.86]). When stratified by years-since-menopause, the ghrelin and hot flush associations were of equal magnitude among women within 10 years-since-menopause (OR [95% CI] =1.86 [1.03-3.36]) and women of more than 10 years-since-menopause (OR [95% CI] =1.75 [0.97-3.19]; P for interaction=0.61). Leptin, adiponectin, and resistin were not associated with experience of hot flushes.

Considering flushing intensity, higher ghrelin concentration was significantly associated with increased odds of mild flushing compared to no flushing (OR [95% CI] =1.35 [1.12-1.62]), but was not associated with mixed intensity flushing compared to no flushing (OR [95% CI] =1.14 [0.99-1.31]) (Table 4). Higher leptin level was significantly associated with decreased odds of mild compared to no hot flushes (OR [95% CI] =0.78 [0.65-0.94]) but not with mixed intensity flushing (OR [95% CI] = 1.01 [0.87-1.16]). Adiponectin and resistin concentrations were not associated with flushing intensity.

TABLE 4. Associations of ghrelin and adipokines with hot flush intensity

Analytes	Flushing intensity	All women			Normal BMI (BMI ≤ 25kg/m ²)			Overweight and obese (BMI > 26 kg/m ²)		
		N	OR	95% CI	N	OR	95% CI	N	OR	95% CI
Ghrelin, pg/mL	None	399	Ref		138	Ref		229	Ref	
	Mild only	154	1.35	1.12-1.62	70	1.11	0.82-1.50	72	1.43	1.11-1.85
	“Mixed	345	1.14	0.99-1.31	128	1.10	0.85-1.41	189	1.23	0.96-1.60
Leptin, ng/mL	None	399	Ref		138	Ref		229	Ref	
	Mild only	154	0.78	0.65-0.94	70	0.58	0.37-0.92	72	0.96	0.72-1.28
	Mixed	345	1.01	0.87-1.16	128	1.15	0.83-1.58	189	1.02	0.82-1.27
Adiponectin, μg/mL	None	399	Ref		138	Ref		229	Ref	
	Mild only	154	0.95	0.79-1.13	70	0.83	0.63-1.11	72	0.93	0.73-1.19
	Mixed	345	1.03	0.89-1.19	128	0.99	0.78-1.26	189	1.04	0.86-1.25
Resistin, ng/mL	None	399	Ref		138	Ref		229	Ref	
	Mild only	154	0.96	0.80-1.15	70	1.17	0.89-1.54	72	0.82	0.64-1.04
	Mixed	345	0.95	0.82-1.09	128	0.92	0.73-1.16	189	0.97	0.82-1.17

Models adjusted for age, education, marital status, and years-since-menopause.

BMI, body mass index.

^aMixture of mild, moderate, and severe.

DISCUSSION

In this sample of healthy postmenopausal women, higher ghrelin concentration was associated with a greater prevalence of self-reported vasomotor flushing, primarily mild hot flushing, adjusted for other correlates of flushing. Women in the highest quartile of ghrelin concentration (>1530 pg/mL) had 1.84 times higher likelihood of report of hot flushes compared to women in the lowest quartile of ghrelin (<929 pg/mL) (Table 2). Although the test for interaction was not statistically significant, there was some suggestion that this ghrelin association was of greater magnitude among overweight and obese women (OR = 2.36). A trend of increasing prevalence of hot flushes across ghrelin quartiles was also significant ($P = 0.02$) among overweight and obese women; however, no such trend was observed among women with ideal BMI ($P = 0.42$; P value for interaction = 0.46). Stratified analysis by time since menopause did not show any striking difference in these associations between early versus late postmenopausal women. In contrast to prior studies reported primarily in perimenopausal and early postmenopausal women, none of the adipokines were associated with flushing experience in our study participants. When we evaluated flushing intensity, higher ghrelin concentration was associated with increased odds of reporting mild flushing compared to no flushing. Higher leptin levels were significantly associated with decreased odds of mild flushing.

Our finding involving ghrelin and hot flushes is novel and intriguing. Ghrelin is a peptide hormone predominantly produced by the stomach cells.²⁰ Ghrelin travels to the central nervous system, crossing the blood brain barrier.²¹ Ghrelin receptors (growth hormone secretagogue receptors, Ghsr) are expressed in the hypothalamus, which on activation serve as a potent stimulant for growth hormone secretion,²² increased food intake, adiposity, and play an important role in energy homeostasis.^{16,23} The vast majority of literature explaining the mechanism of vasomotor flushing suggests that hot flushes result from a thermoregulatory imbalance in the hypothalamus. Menopause-induced changes in the thermoregulatory neurons result in narrowing of the thermoneutral zone, along with reduction of the temperature threshold for a compensatory vascular response.²⁴⁻²⁶ The molecular basis of this mechanism is the group of neurons in the hypothalamus including Kisspeptin, Neurokinin B (NKB), and Dynorphin, collectively known as KNDy neurons, which regulate puberty, fertility, and energy homeostasis.^{27,28} Animal and human studies have shown significant hypertrophy of the hypothalamic region after menopause along with a striking increase in NKB and Kisspeptin gene expression in that region.²⁹ Based on their initial observation of marked increases in hypothalamic NKB gene expression after menopause, Rance and Young⁷ proposed that hypothalamic neurons containing estrogen receptor, and NKB mRNA regulate the negative feedback mechanism of estrogen in women. A substantial body of accumulating evidence suggests a pivotal role of KNDy neurons in generating the hot flush.⁶ Further evidence from an experimental study showed coexpression of

Ghsr and Kiss1r in subsets of preoptic and arcuate nucleus, particularly in ovariectomized mice treated with estradiol, suggesting a modifying role of estradiol on the upregulation of Kiss1 neurons by ghrelin at the hypothalamic level.³⁰ On the contrary, a handful of experimental studies in animals and young women suggest ghrelin administration may decrease pituitary gonadotropin secretion,³¹⁻³³ contradicting the findings above. Clearly, more studies are warranted to unravel the role of ghrelin on gonadotropin regulation, in particular with respect to menopause and associated flushing.

In addition to this central component, mechanisms of vasomotor flushing include peripheral components such as adiposity. The impact of BMI and adipose tissue on hot flushes has been of long-standing interest. Supporting the thermoregulatory hypothesis, multiple reports indicated that higher BMI and adiposity is associated with greater prevalence of hot flushes,^{1-4,34} as increased fat content is hypothesized to interfere with body heat dissipation.³⁵ However, weight gain in postmenopausal women was protective for hot flushes due to relatively higher estrogen levels from aromatization of androstenedione to estradiol in adipose tissue.^{11,36} In the current study, we found significantly lower BMI in women reporting hot flushes compared to those who did not experience flushing (Table 1).

It is interesting to note that, despite the inverse association between BMI and hot flushes, the ghrelin and hot flush association was more pronounced among overweight and obese compared to women of ideal BMI. Although the interaction was not statistically significant, the ORs are notably different between normal versus overweight and obese women (ORs [95% CIs], 1.24 [0.54-2.86] vs 2.36 [1.28-4.35]). Our data show an inverse association between ghrelin and BMI (age adjusted β -estimate [SE] = -0.005 [0.0003], P value < 0.0001), which is consistent with literature reporting significantly lower ghrelin concentration in obese individuals compared to matched lean controls.³⁷ Further research is warranted to understand if and how BMI modifies the ghrelin and hot flush association we observed in these data.

Adipose tissue-derived cytokines, collectively known as adipokines, have been of research interest to further explain the contribution of body fat to vasomotor flushing. We found no association between leptin, adiponectin, resistin, and flushing. A subcohort SWAN study reported significantly higher odds of hot flushes with higher leptin concentration, and lower concentration of adiponectin.¹¹ Notably, these adipokine associations with flushing were evident in pre-/early perimenopause but not in late peri/post-menopause. A small cross-sectional study among nonsmoking white women participating in the MWHs reported a statistically significant association between higher leptin levels and flushing (measured as any flushing within the last 30 days, and duration).¹⁰ Of note, both SWAN and MWHs included premenopausal or early perimenopausal women and followed them through the menopausal transition. Our study participants were postmenopausal, with many being more than

10 years postmenopause, which may explain different findings in these studies. Notably, a lack of association between adipokines and hot flushing among late-perimenopausal and postmenopausal women in the SWAN study supports our study findings.

When hot flush intensity (mild only vs mixture of mild, moderate, and/or severe) was considered, we found women with higher leptin levels had significantly lower odds of mild only hot flushes. Leptin has been shown to be an important regulator of energy balance in humans.³⁸ Leptin supplementation suppresses food intake,³⁹ and leads to weight loss⁴⁰ in healthy humans. There is evidence of an adverse effect of leptin on ovarian function,⁴¹ which supports the inverse correlation between leptin and estradiol reported among pre-/perimenopausal women participating in the Midlife Health Study.¹⁰ On the contrary, we previously reported a significant positive correlation between leptin and estrogen levels in the same postmenopausal women reported in this current study.¹⁷ In addition, Sowers et al⁴² reported that serum leptin concentrations increase as women, nonobese in particular, progress from pre- to postmenopausal stages. Although the underlying reason for a differential association between leptin and estradiol concentration by menopausal status is not well understood, it is possible that the inverse association of leptin on mild hot flushes in our study participants could be mediated by the relatively higher estradiol levels associated with higher leptin. The null associations of leptin as well as ghrelin with mixed intensity hot flushes are intriguing. It is possible that the mechanisms of mixed intensity hot flushes involve different and possibly more complex components in addition to these peptide hormones. These proposed explanations need further investigation.

To our knowledge, our study is the first to report higher serum ghrelin concentration as a correlate of hot flushes in a considerably large sample of generally healthy postmenopausal women. This sample equally represented early and late menopausal groups based on their years-since-menopause. The limitations of this study include the cross-sectional design and self-reported hot flush information. Therefore, recall bias could be a potential threat to the internal validity. However, there is no apparent reason for women to recall flushing experience disproportionately based on their unknown ghrelin and adipokine concentrations.

Our results have significant clinical and public health implications. Although vasomotor flushing is experienced by over 70% of women during the menopausal transition, and a considerable proportion of them continue experiencing those symptoms late into postmenopause, the factors related to hot flushes are not fully identified. Vasomotor flushing is a complex interplay between central and peripheral components. An abrupt reduction in estradiol and rise in gonadotropin clearly drive the physiology of menopause; hence those are the consistent predictors of hot flushes.^{34,43,44} However, the fact that not all women experience hot flushes is indicative of other conceivable drivers contributing to flushing physiology. In addition to these peripheral contributors, the

hypothalamus plays a central role in thermoregulation. Considering the physiology of flushing, factors that can potentially trigger the thermoregulatory zone in the hypothalamus and factors interfering with heat dissipation are potential mechanistic contributors to vasomotor flushing. Ghrelin is a prime candidate for a contributor to hot flushes, as ghrelin receptors are expressed on the hypothalamus and ghrelin is produced in the stomach as well as in the hypothalamus.⁴⁵ A recent randomized, double blinded, phase 2 trial testing a neurokinin 3 receptor antagonist showed promising results on reducing hot flushes.⁴⁶ This elucidates the possibility of therapeutic interventions targeting ghrelin and leptin receptors in the hypothalamus for reduction of obesity as well as vasomotor flushing.

CONCLUSIONS

Hot flushes are a common menopausal symptom experienced by most women going through the menopausal transition and a considerable proportion of women continues experiencing hot flushes late into postmenopause. Although lack of estrogen is the primary cause of vasomotor symptoms, understanding the specific mechanism of flushing is critical in order to find an effective therapeutic remedy. Our research sheds light on the contribution of ghrelin in hot flushes. More investigation is warranted for further understanding of the mechanism of vasomotor symptoms that compromise the quality of life of menopausal women.

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