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Serum Leptin Levels, Hormone Levels and Hot Flashes in Midlife Women

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Abstract

Objective—This study examined the associations between serum leptin levels, sex steroid hormone levels, and hot flashes in normal weight and obese midlife women.

Design—Cross-sectional study.

Setting—University clinic.

Patient(s)—Two-hundred and eleven Caucasian women who were non-smokers between the ages of 45 and 54 years with a body mass index (BMI) of <25 kg/m2 or ≥30 kg/m2 were included. Intervention(s): Participants completed a questionnaire and provided fasting blood samples, which were used to measure leptin and sex steroid hormone levels. Correlation and regression models were performed to examine associations between leptin levels, hormone levels, and hot flashes.

Main outcome Measure—Serum leptin levels

Result(s)—Leptin levels were associated with BMI (r=0.75, p<0.0001). Leptin levels were associated with 'ever experiencing hot flashes' (p=0.04), hot flashes within the last 30 days, (p=0.03), and duration of hot flashes (>1 year, p=0.03). Leptin was positively correlated with testosterone, free testosterone index, and free estrogen index; meanwhile, leptin levels were

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inversely associated with levels of sex hormone binding globulin (SHBG). In women with a BMI≥30 kg/m2, leptin levels were no longer correlated with testosterone levels.

Conclusion(s)—Based on this data, serum leptin levels are associated with the occurrence and duration of hot flashes in midlife women; albeit, no correlation was found between leptin and serum estradiol.

Introduction

It is estimated that approximately between 40% (1,2) to 100% (3) of women experience hot flashes during the menopausal transition. Although the intense heat, flushing and sweating that occur during a hot flash may disrupt a woman's quality of life (4–6), little is known about risk factors for hot flashes. Previous studies have examined potential risk factors for hot flashes and found that smoking, and low levels of estrogens increase the risk of hot flashes (4,7,8), whereas moderate alcohol use decreases the risk of hot flashes (9,10). Interestingly, studies have also shown that obesity (BMI ≥ 30kg/m^2) is associated with an increased risk of experiencing hot flashes (4,7,11–13). Despite the magnitude of this public health problem, the reasons that obesity is associated with an increased risk of hot flashes are unclear. Overlie et al. (14) showed that women who developed hot flashes entered the menopausal transition with high estrogen levels that dropped over time, but saw that women with low to average estrogen levels did not develop hot flashes. Some studies suggest that high adiposity in obese women may serve as a potent insulator (15), thereby inhibiting heat loss and increasing levels of vasomotor symptoms (16,17). It is also possible that obesity is associated with hot flashes through a mechanism that involves alterations in cytokines, including leptin, which lead to thermoregulatory dysfunction (18,19). Leptin is a highly conserved cytokine-like protein that regulates energy balance through its actions in the brain on appetite and energy expenditure. It is expressed by adipocytes in proportion to body fat mass. Leptin is secreted in a pulsatile manner and has a diurnal rhythm, with a peak at night and a nadir in the morning. The diurnal variations in leptin are absent in amenorrheic women athletes, who do not experience hot flashes (20). Sowers et al. (21) investigated the menopause stage with adipocytokines, including leptin, adiponectin and resistin in obese and non-obese women. reported that leptin levels increased from the premenopause stage to the postmenopause stage in non-obese women.

Differences in leptin levels may play a role in the pathophysiology of hot flashes and may help elucidate the reason why women specifically in the menopausal transition wake during the night soaked in sweat after experiencing hot flashes. Thus, we hypothesized that serum leptin levels are associated with occurrence, frequency or severity of hot flashes in women. Further, since high leptin levels are known to suppress ovarian steroid production (22,23), this study was also designed to determine whether leptin levels were associated with levels of sex steroid hormone levels. A better understanding of the risk factors for hot flashes could eventually lead to the design of better therapies for hot flashes and improved counseling of our patients.

Materials and Methods

Study Population and Study Design

The data for the current study were collected as part of the Midlife Health Study. The Midlife Health Study which was designed to evaluate risk factors for hot flashes in midlife women aged 45 to 54 years, living in Baltimore and its surrounding counties. All participants in the study (N=639 women) gave written informed consent according to procedures approved by the Johns Hopkins University and University of Maryland School of Medicine Institutional Review Boards.

Women were included in this analysis (N=211) if they were Caucasian, aged 45 to 54 years, were non-smokers, not pregnant, had an intact uterus and both ovaries, at least 3 menstrual periods in the previous 12 months, and a BMI <25 kg/m^2 (normal weight) or ≥30 kg/m^2 (obese). In order to exclude race and smoking status as confounding variables, this analysis focused on women who were Caucasian non-smokers.

The detailed methods for the Midlife Health study have been described elsewhere (7–9,24). Briefly, participants presented to clinic fasting between 8:30 and 10:00am. Subsequently, each participant completed a detailed 26-page survey, including questions regarding demographic information, reproductive history, menstrual cycle characteristics, hormonal contraceptive use, menopausal symptoms, hormone replacement therapy use, medical and family history, health behaviors (smoking, vitamin use, and eating habits), alcohol intake, and hot flash history. If individuals answered yes to the question, “Have you ever had hot flashes?” they were categorized as ‘women with hot flashes’ and those who answered ‘no’ were categorized as ‘women without hot flashes’. The hot flash score refers to individuals who never felt hot flashes, felt the hot flashes were bothersome, moderately or very bothersome. The initial inclusion criteria ensured that the women could not be pregnant, not be taking any hormone replacement therapy, herbal supplements, or hormonal contraception and have no personal history of cancer of the reproductive organs. Waist circumference was measured at the narrowest part of the waist. Hip circumference was measured at the fullest part of the hips. The participant was weighed without shoes in street clothing to the nearest 0.05 lb, rounding down, on a calibrated scale. Her height was measured without shoes to the nearest 0.5 in., rounding down, with a standard stadiometer. BMI was calculated using the National Institutes of Health on-line BMI calculator (25).

Hormone Assays

Serum concentrations of leptin, estradiol, estrone, testosterone, androstenedione, dehydroepiandrosterone-sulfate (DHEA-S), progesterone, and sex hormone binding globulin (SHBG) were measured using enzyme-linked immunosorbent assays (ELISA). ELISA kits for estradiol, testosterone, androstenedione, and DHEA-S were obtained from Diagnostic Systems Laboratories, Inc. (Webster, TX). ELISA kits for estrone, progesterone, and SHBG were obtained from American Laboratory Products Company (Windham, NH). Using the manufacturers’ instructions, each sample was run in duplicate without knowledge of the participants’ hot flash status. In addition, positive controls containing known amounts of leptin, estradiol, estrone, testosterone, androstenedione, DHEA-S, progesterone, or SHBG were included in each batch.

According to the manufacturer, cross-reactivities between measured hormones were as follows: DHEA-S: estrone-sulfate 0.88%; estradiol: equilinin 2.73%, estrone 1.38%, estrone-sulfate 1.23 %, estrone-glucuronide 0.36%; estrone: estrone-3-sulfate 4.9%, estradiol 2.2%, estrone–gulcuronide 1.2%, estradiol-3-glucuronide 0.14%; testosterone: 5α-dihydrotestosterone 6.6%, 5-androstane-3β,17β-diol 2.2%, 11-oxo-testosterone 1.8%, ASD 0.9%, 5β-dihydrotestosterone 0.6%, 5β-androstane-3β,17β-diol 0.5%, 17β-estradiol 0.4%, 5α-androstane-3α-ol-17-one. The minimum detection limits and intra-assay coefficients of variation were as follows: leptin 0.0ng/mL ± 0.15%, estradiol 7pg/ml, 3.3 ± 0.17%; estrone 10pg/ml, 4.8 ± 0.25%; testosterone 0.04ng/ml, 2.2 ± 0.56%; androstenedione 0.03ng/ml, 2.5 ± 0.60%; DHEA-S 15ng/ml, 1.9 ± 0.63%; progesterone 0.1ng/ml, 2.1 ± 0.65%; and SHBG 0.1nmol/L, 2.4 ± 0.67%. No leptin, estradiol, estrone, testosterone, androstenedione, DHEA-S, or SHBG samples were below the limit of detection.

Free estradiol index (FEI) was calculated to estimate the amount of estradiol unbound by SHBG and therefore thought to be biologically active. A ratio of total estradiol to SHBG was calculated using a conversion factor to change pg/ml of estradiol to nmol/L: 100 × (total

estradiol × 0.003671)/SHBG (26). The free testosterone index (FTI) was also estimated using a conversion factor to change ng/ml of testosterone to nmol/L: 100 × (total testosterone × 3.467)/SHBG (27).

Statistical Analyses

Prior to conducting this sub-study, we calculated that a sample size of 100 per group (women with and without hot flashes) would provide 80% power to see a 35% difference in serum leptin between women with and without hot flashes (two-tailed alpha of 0.05; based on mean and standard deviation values reported by Considine et al (27). Our final sample sizes of 119 women with hot flashes and 92 women without hot flashes provided a similar level of power to observe the 35% difference in leptin levels between the two groups.

Demographic and health habit characteristics of women with and without hot flashes were compared using chi-square tests for categorical variables and Student's t-test for continuous variables. Estradiol, estrone, and leptin data were log-transformed because the data for these variables were not normally distributed. Correlations between leptin and hormone levels were carried out using Spearman rank correlation. The associations between the hot flash variables and the log-transformed leptin variable adjusted for age and days since last menstrual cycle were assessed using generalized linear models (PROC GLM in SAS).

Results

Characteristics of the participants with and without hot flashes are presented in Table 1. The mean age of the participants was 48.8±2.4 years. The patients were divided according to their BMI: <25 kg/m^2 (N=119) or ≥30 kg/m^2 (N=92). Women with hot flashes were more likely to be older (0.02), compared to women without hot flashes. In addition, women with hot flashes were more likely to have a higher BMI (p=0.003) and larger waist circumference (p=0.005) than women without hot flashes. Further, 56% of the participants with hot flashes had a waist circumference greater than 35 inches, whereas only 35.9% of women without hot flashes had a waist circumference greater than 35 inches. As previously reported (7), mean estradiol concentrations were lower in women with hot flashes as compared to those without hot flashes (p=0.0004). Similarly, mean estrone levels were also lower in women with hot flashes compared to those without hot flashes (p=0.003). However, there was no statistically significant difference in marital status, education, prior pregnancy or alcohol intake between the groups (Table 1). All of the women in this analysis were Caucasian non-smokers, in order to reduce confounding variables.

As shown in Table 2, serum leptin levels were significantly associated with BMI (r=0.75, p<0.0001 Table 3 depicts the analysis of the associations between leptin levels and hot flash questionnaire responses adjusted for age and days since last menstrual period. There was a significant association between leptin levels and the occurrence of hot flashes (p=0.04). Leptin levels were associated with ever experiencing hot flashes (p=0.04), hot flashes within the last 30 days, (p=0.03), and duration of hot flashes (>1 year, p=0.03). However, severity of hot flashes and frequency of hot flashes, as well as hot flash score were not associated with leptin levels.

Correlations between leptin levels and hormone levels are shown in Table 4. Leptin levels were inversely correlated with levels of sex hormone binding globulin (SHBG) (p<0.0001), and positively correlated with testosterone levels, free estrogen index (FEI) (p<0.0001) and free testosterone index (FTI) (p<0.0001). Further, in women with a BMI ≥30 kg/m2, leptin levels were inversely correlated with SHBG (p=0.02) and positively correlated with FTI (p<0.0001) and FEI (p<0.0001), but were no longer correlated with testosterone (p=0.4). Although leptin levels were associated with hot flashes, leptin levels were not correlated

with estradiol or estrone levels. Furthermore, leptin levels were not correlated with androstenedione, progesterone, or DHEA-S levels in this study sample.

Discussion

Previous studies have shown that a high BMI is associated with increased occurrence of hot flashes in midlife women (12,29–32). The mechanism by which a higher BMI increases the odds of hot flashes is unclear. Previous studies report a significant correlation between measures of body fat (BMI, % body fat) and circulating concentrations of leptin in adults (27,33). Leptin is known to mediate the formation of estrogens from circulating androgen precursors and to affect thermoregulation (15,18,19). Since leptin is associated with high BMI, low estrogen levels, and thermo-dysregulation, which are known risk factors for hot flashes, this study determined whether leptin was associated with obesity, hot flashes, and low steroid hormone levels in midlife women.

The data from this study indicate that serum leptin levels are associated with high BMI, as well as, ever experiencing hot flashes, the experiencing of hot flashes within the last 30 days and a longer duration of hot flashes in midlife women. Because of these findings, we examined whether leptin was associated with hormone levels. Consistent with other reports, the results indicate that leptin levels are inversely correlated with SHBG (34,35), but leptin levels are positively correlated with FTI and FEI. Albeit, FTI and FEI are calculated using SHBG, there is evidence that obesity was associated with a decreased level of SHBG (36–40). In adipose stromal cells, high levels of leptin (100ng/mL) increase aromatase gene promoter activity (41). As such, one would expect that the higher leptin levels would lead to higher estrogens, but our results show the mean estradiol and estrone levels were lower in women with hot flashes as compared to those without hot flashes. These findings confirm a previous report evaluating the interrelationships of serum estradiol, estrone, adioposity and leptin in post-menopausal women (42). Castracane et al. (42) reported that serum leptin levels are not associated with increases in estradiol and estrone. To the best of our knowledge, there are no studies examining the correlation between leptin levels and FTI and FEI.

The role of leptin in the mechanism leading to hot flashes may involve leptin's action in the brain on appetite and energy expenditure. Luheshi et al. (43) reported that the injection of leptin peripherally increase core body temperature. According to previous studies, an increase in core body temperature precedes hot flashes in postmenopausal women (44,45). Leptin acts on specific receptors in the hypothalamus, a region associated with energy expenditure (46,47). The modification of serum leptin levels and leptin receptor expression in hypothalamus and adipose tissue by estrogen status identifies a close cross-talk between central and peripheral tissues in the regulation of body fat mass and weight (48). Though our study did not show a correlation between leptin and estradiol, the alterations in reproductive hormone levels, including the declining estrogen concentrations has long been considered a factor in the etiology of hot flashes.

Previous investigators have shown a relationship between leptin and estrogen production. In a study using rats, leptin impaired the sensitizing effect of insulin-like-growth factor-1 (IGF-1) on follicle stimulating hormone (FSH)-dependent estradiol synthesis by granulosa cells (49). This study also concluded leptin can act directly on the ovarian granulosa cell to selectively decrease estradiol production, but not progesterone production. Of note, in the clinical setting, when administering a selective estrogen-receptor modulator, raloxifene, a notable increase in hot flashes is a significant side effect. Cakmak et al. (47) report that raloxifene increases serum leptin levels, while IGF-1 levels are decreased. Furthermore, in ovariectomized rats, modification of estrogen levels using raloxifene led to a time dependent

elevation of serum leptin levels as estrogen was withdrawn (48). The interplay between leptin and estrogens need further investigations.

Our study showed an association between leptin and hot flashes and an inverse correlation with SHBG, yet this could not be explained by an alteration in estradiol. The nightly slowing of luteinizing hormone (LH) pulses and the fall in metabolic rate is associated with the timing of the leptin peak (50,51). There is some speculation that fluctuations in glucose may initiate the cascade of events that leads to a hot flash. Basal leptin secretion by adipocytes may require the availability of glycolytic substrates (52). In rat studies, increased leptin messenger RNA expression correlates with an increased uptake and utilization of glucose by adipocytes (53). Hyperinsulinemia can influence diurnal leptin release (54). One study reported that repeated daytime postprandial high insulin levels led to an increased release of leptin, which subsequently peaks during the night; meanwhile, the nocturnal postabsorptive diminution in insulin levels can cause a decline in leptin that manifests in the early morning hours (54). A better understanding of the specific signals and energy fuels that trigger the nocturnal rise in leptin and significant changes in the profile of LH pulses may further our understanding of the pathophysiology of hot flashes.

In interpreting the results of this study, it is important to consider its strengths and limitations. A major strength of this work is that the study was specifically designed to examine hot flashes in midlife women; therefore, a very detailed hot flush history was obtained from each participant. In addition, this study is a novel examination of hot flashes and their association with serum leptin levels. One of the limitations of this study is that we only collected one blood sample from each participant and thus, only one sample was available to measure leptin and sex steroid hormone levels. In addition, the presence of cross-reactivities cannot be avoided, but chromatographic procedures using gas chromatography–mass spectrometry, liquid chromatography (LC)–mass spectrometry, and LC –tandem mass spectrometry are superior assays for sex steroid levels. Further, many factors can contribute to leptin levels, including acute caloric intake, fat mass, adipocyte size, fat distribution, insulin levels, glucocorticoids, and cytokines (54,55). In an attempt to minimize some of this variability, women were asked to fast before blood collection, all samples were collected in the morning between 8:00 and 10:00am, and in the analysis we adjusted for age and time since the beginning of last menstrual period. We elected to perform a cross-sectional analysis because a prospective design requires more resources and effort on the part of the study participants. Furthermore, a larger sample size would have increased the power of the study.

In summary, based on the results from this population-based cross-sectional study, leptin may play a role in the pathophysiology of hot flashes. Behavioral modifications including weight loss may be important when counseling patients with significant hot flashes in midlife women. Future investigations are warranted to confirm these findings, including other adipocytokines as well.

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Table 1

Characteristics of study participants and controls

Variable	With Hot Flashes	Without Hot Flashes	p-value
Sample size	109	92	
Participant Characteristics			
Age (years), mean (SD)	49.2 (2.2)	48.4 (2.5)	0.02
Race			
White (n, %)	109 (100%)	90 (100%)	0.1
Marital Status			
Single	10 (9.2%)	16 (17.4%)	0.2
Married	75 (68.8%)	58 (63.0%)	
Other	24 (22.0%)	18 (19.6%)	
Education			
High school degree	21 (19.3%)	9 (9.8%)	0.1
Some college	35 (32.1%)	28 (30.4%)	
College degree	53 (48.6%)	55 (59.8%)	
BMI (kg/m2)			
<25.0	46 (42.2%)	58 (63.0%)	0.003
≥ 30.0	63 (57.8%)	34 (37.0%)	
Waist circumference			
<30 inches	38 (34.9%)	53 (57.6%)	0.005
30–34 inches	10 (9.2%)	6 (6.5%)	
35+ inches	61 (56.0%)	33 (35.9%)	
Ever pregnant			
Yes	93 (85.3%)	75 (81.5%)	0.6
Prior HRT			
Yes	5 (4.6%)	2 (2.2%)	0.4
Current alcohol drinker			
Yes	68 (62.4%)	67 (72.8%)	0.1
Estradiol levels, pg/mL, geometric mean (range)	79.3 (2.9, 536.4)	121.4 (13.0, 612.0)	0.0004
Estrone levels, pg/mL, geometric mean (range)	115.1 (7.6, 365.0)	154.4 (31.9, 508.0)	0.003

Table 2

Association between BMI and unadjusted geometric mean leptin levels

BMI (kg/m^2)	Leptin levels (geometric mean)	95% LL	95% UL	p-value
<25	5.99	5.33	6.73	<0.0001
>30	41.08	36.40	46.36	

Table 3

Associations between leptin levels and hot flashes – adjusted for age and days since last menstrual period

	Leptin levels (age-adjusted geometric mean)	95% LL	95% UL	p-value
Ever experienced hot flashes				0.04
No	12.70	10.03	16.08	
Yes	17.62	14.19	21.87	
Experienced hot flashes w/in last 30 days				0.03
Never experienced hot flashes	12.63	9.99	15.97	
No (women with ever hot flashes)	13.41	9.25	19.43	
Yes	20.30	15.56	26.49	
Severity of hot flashes				0.1
Never experienced hot flashes	12.71	10.03	16.10	
Mild	16.18	11.60	22.56	
Moderate/Severe	18.68	14.04	24.85	
Frequency of hot flashes				0.1
Never experienced hot flashes	12.64	9.95	16.04	
Monthly	15.85	11.45	21.94	
At least weekly	18.97	13.72	26.23	
Duration of hot flashes				0.03
Never experienced hot flashes	12.72	10.05	16.11	
<1 year	13.17	8.89	19.53	
>1 year	20.31	15.54	26.55	
Hot flashes score				0.1
Never experienced hot flashes	12.63	9.96	16.03	
Least bothersome	15.81	11.82	21.13	
Moderately/Very bothersome	19.93	13.97	28.45	

Table 4

Correlations between leptin levels and sex steroid hormone levels

	All women		BMI ≥ 30 kg/m2	
	r	p-value	r	p-value
Estradiol	−0.03	0.7	0.12	0.2
Estrone	−0.03	0.7	0.14	0.2
Testosterone	0.29	<0.0001	0.10	0.4
Androstenedione	0.03	0.6	−0.07	0.5
Progesterone	−0.10	0.2	0.11	0.3
DHEA-S	0.03	0.7	−0.05	0.7
SHBG	−0.66	<0.0001	−0.24	0.02
FTI	0.63	<0.0001	0.26	0.01
FEI	0.39	<0.0001	0.25	0.01