

Circulating Leptin and Stress-induced Cardiovascular Activity in Humans

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Obesity is associated with an elevated risk of hypertension and cardiovascular disease. The adipocyte hormone leptin, which stimulates energy expenditure in animals by activating the sympathetic nervous system (SNS), is believed to play a role in this association. However, evidence in humans remains sparse. We investigated the relationship between circulating leptin and cardiovascular and inflammatory responses to acute psychological stress in humans. Participants were 32 men and 62 women aged 18–25 years. Cardiovascular activity was assessed using impedance cardiography at baseline, during acute laboratory stress, and during a 45-min recovery period. Plasma cytokines were measured in blood drawn at baseline and 45-min poststress. In women only, baseline plasma leptin was significantly associated with stress-induced changes in heart rate ($\beta = 0.53$, $P = 0.006$), heart rate variability (HRV) ($\beta = -0.44$, $P = 0.015$), and cardiac preejection period (PEP) ($\beta = -0.51$, $P = 0.004$), independent of age, adiposity, and smoking. Women's plasma leptin levels also correlated with stress-induced elevations in the proinflammatory cytokine interleukin-6 (IL-6) ($\beta = 0.35$, $P = 0.042$). Circulating leptin is an independent predictor of sympathetic cardiovascular activity, parasympathetic withdrawal, and inflammatory responses to stress in women. Because cardiovascular and inflammatory stress responses are predictive of future cardiovascular disease, leptin may be a mechanism mediating the adverse effects of stress and obesity on women's cardiovascular health.

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INTRODUCTION

The incidence of hypertension and cardiovascular disease is significantly elevated in obese individuals (1). This is most apparent in women, where the relationship between adiposity and cardiovascular risk is stronger than for men (2,3). One factor thought to play a role in this effect is the adipocyte hormone leptin. Secreted into the blood stream in proportion to adipose tissue mass, leptin regulates energy balance through binding to receptors on specific hypothalamic nuclei and reducing food intake as well as increasing energy expenditure (4). *Ob/ob* mice lacking the leptin gene are profoundly obese, whereas markedly elevated circulating levels of leptin in obese humans and animals suggest a form of “leptin-resistant” obesity (4). Notably, women have significantly higher circulating levels of leptin than men of comparable body fat mass (5).

Animal evidence suggests that one of the ways in which leptin increases energy expenditure is through activating the sympathetic nervous system (SNS). Acute intravenous or intracerebroventricular leptin infusion increases sympathetic nerve activity to thermogenic and nonthermogenic organs of rodents, including interscapular brown adipose tissue, kidney, hindlimb, and adrenal gland (6). Chronic systemic, as well as central, leptin infusion raises circulating catecholamine

concentrations and stimulates heart rate and arterial blood pressure (BP) in animals (6,7). These chronic effects of leptin are abolished by α - and β -adrenergic blockade, indicating that they are mediated by sympathetic adrenergic activation (6,7). Furthermore, genetically obese mice with hyperleptinemia develop hypertension, whereas leptin-deficient *ob/ob* mice display lower arterial BP than lean controls (8).

The evidence relating leptin to sympathetic nervous activity in humans is less clear. A number of cross-sectional clinical studies have reported positive correlations between circulating leptin levels and sympathetic activation indexed by BP, heart rate variability (HRV), and renal sympathetic outflow in lean and obese normotensive humans (9–11). Plasma leptin levels were also found to correlate with heart rate in patients with essential hypertension (12). However, interventions examining the cardiovascular effects of leptin infusion in humans have been inconclusive. One study found that low-dose leptin administration reversed the decline in SNS activity and urinary epinephrine levels associated with weight loss in men (13). In contrast, others found no effect of administering physiological or pharmacological doses of exogenous leptin on autonomic activation or urinary catecholamines in humans (14–16).

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The effects of leptin in humans may be more apparent under conditions of sympathetic activation, induced by factors such as psychological stress. Psychological stress stimulates the SNS and is a recognized risk factor for hypertension and cardiovascular disease (17). Acute stressors upregulate circulating levels of a number of inflammatory markers implicated in obesity-related hypertension in humans, including the cytokine interleukin-6 (IL-6) (17). Cross-sectional studies also report positive associations between circulating levels of these markers and leptin levels in lean and obese humans (18,19). We hypothesized that people with elevated circulating leptin levels would have greater cardiovascular and inflammatory responses to psychological stress, and set out to investigate this in a healthy sample of men and women.

METHODS AND PROCEDURES

Participants

For the purpose of this study, 62 women and 32 men, aged 18–25 years, were recruited from University College London. Volunteers were screened by structured interview to ensure that they were healthy, had no previous history of any relevant physical or mental illness, and were not taking any medication. They were instructed not to consume caffeinated beverages or alcohol and to refrain from excessive exercise, during the 12 h prior to the session. They were also asked not to take aspirin, ibuprofen, or antibiotics for 10 days prior to testing, to avoid a high-fat breakfast, and were provided with a standardized low-fat lunch prior to the session. The lunch consisted of a vegetable couscous followed by a fruit salad and fresh orange juice. All participants gave their written informed consent, and the study was approved by the University College London/University College London Hospital Committee on the Ethics of Human Research.

Anthropometric measures

Measures of participants' weight, height, and waist circumference were obtained using standardized methods. Body weight was measured to the nearest 0.1 kg, and height was measured to the nearest 0.1 cm. Waist circumference was measured horizontally midway between the lowest rib and iliac crest. BMI was calculated as body weight in kilograms divided by height in metres squared. Body fat mass was estimated using a Bodystat 1500 bioelectrical impedance body composition analysis device (Bodystat, Douglas, Isle of Man). The Bodystat technology has been scientifically validated in both its accuracy of measurement of impedance *in vivo* and in the application of its own unique regression equation to determine body composition (20). Percentage fat was calculated as fat weight divided by total (fat + lean) body weight. Relationships between central adiposity and cytokine stress responses in the women from this study have been described elsewhere (21).

Cardiovascular measures

Heart rate and HRV were assessed using impedance cardiography (ICG; VU-AMS, Amsterdam, Holland) as described previously (22). BP was measured at baseline using an electronic sphygmomanometer (A & D UA779, Tokyo, Japan). HRV was calculated as the root mean square of successive R-R interval differences. A reduced root mean square of successive R-R interval differences indicates a shift in cardiac sympathovagal balance toward sympathetic control over the rhythm of the heart (23). Cardiac sympathetic drive was indexed by the cardiac pre-ejection period (PEP) (24). One-minute ensemble averages were derived for the ICG for each minute of tasks and averaged. PEP was defined as the interval between R-wave and B-point plus a fixed Q-R interval of 48 ms. Cardiovascular data for the study were collected continuously and then averaged over specified 5-min trials.

Laboratory mental stress testing

Participants were tested individually in the afternoon in a temperature-controlled laboratory. Anthropometric measures were taken and the impedance cardiogram was fitted for continuous assessment of cardiovascular activity. A venous cannula was then inserted in the lower arm for blood sampling and participants were left to relax for 30 min. VU-AMS recordings during the last 5 min of the rest period constituted the baseline trial for heart rate, HRV, and cardiac PEP, and a blood sample drawn at the end of the rest period was used for assessment of baseline plasma levels of leptin and IL-6. Participants then undertook two 5-min behavioral tasks, administered under time pressure. The first was a computerized color-word interference task, involving the successive presentation of target color words printed in an incongruous color. The task was to press a computer key that corresponded to the position at the bottom of the screen of the name of the color in which the target word was printed. In the second task, participants were presented with a hypothetical scenario in which they had been wrongly accused of shoplifting, and were instructed to give a speech in their defense. They were told that their speech would be video recorded and later judged for efficacy and fluency. At the end of the tasks, participants rested for a further 45 min. Five-minute VU-AMS recordings of cardiovascular activity were completed during each of the tasks and then at 10–15, 25–30, and 40–45 min post-task. A second blood sample was drawn at 45-min posttask for the assessment of IL-6. Participants were provided with water but were not allowed to eat during the session.

Cytokine assays

Whole blood (10 ml) was drawn using a 21-gauge butterfly needle into Vacutainer tubes containing EDTA as anticoagulant, then centrifuged immediately at 1,250×g for 10 min at room temperature. Plasma was removed, aliquoted, and frozen at –80°C prior to analysis. Plasma IL-6 concentrations were assessed by a high-sensitivity two-site ELISA from R and D Systems (Oxford, UK). The limit of detection of this assay was 0.09 pg/ml, with intra- and inter-assay coefficients of variation of 5.3 and 9.2%. Plasma leptin concentrations were determined by a commercial ELISA from Ray Biotech at Insight Biotechnology (Middlesex, UK). The limit of detection of this assay was 6 pg/ml, with intra- and inter-assay coefficients of variation of <10 and 12% respectively.

Statistical analyses

The distribution of plasma leptin concentration was skewed, so data were transformed with a square root transformation before analysis. Root mean square of successive R-R interval differences data were also skewed, and were log transformed; however, raw values are presented for comparability with other studies. The cardiovascular and inflammatory stress responses were compared in men and women using repeated measures analysis of variance, with the Greenhouse Geisser correction for degrees of freedom where appropriate. Associations between plasma leptin and cardiovascular and cytokine stress responses were analyzed using multiple regression on stress reactivity, computed as the change between baseline and stress levels, in men and women separately. Age, smoking status, BMI, waist circumference, percentage body fat, and baseline levels of the relevant dependent variable (heart rate, HRV, etc.) were included in the models as covariates because they are potentially associated both with leptin and cardiovascular reactivity. Results are presented as standardized regression coefficients (β) with standard errors (s.e.). Associations between leptin and reactivity were illustrated by displaying the mean cardiovascular and inflammatory responses of people in the lower, middle, and higher tertiles of plasma leptin, adjusted for covariates.

RESULTS

Characteristics for men and women participating in this study are presented in **Table 1**. Participants were relatively young with an average age of 21, and all were normotensive. The

majority were White nonsmokers, and all had glycated hemoglobin levels in the normal range. They were not overweight on average. However, there were large individual differences in adiposity measures. BMI ranged from 18.4 to 34.0 kg/m² in women and 19.8 to 28.5 kg/m² in men, and percentage body fat ranged from 10.1 to 40.5% in women and 4.8 to 22.4% in men. Men generally weighed more than women, and had larger waists, whereas women had higher percentage body fat and higher circulating leptin. Men had a higher systolic BP. There were no significant gender differences in BMI, age, ethnicity, smoking, glycated hemoglobin levels, or other baseline measures including plasma IL-6, diastolic BP, heart rate, HRV, or cardiac PEP.

Baseline plasma leptin levels

Individual participants varied widely in absolute leptin levels, ranging from 5.7 to 205.5 ng/ml, with a mean concentration of 35.7, s.d. 22.0 for women, and from 0.1 to 9.7 ng/ml, with a mean concentration of 2.3, s.d. 2.0 for men. The coefficient of variation was 0.62 for women, and 0.87 for men, indicating that variability was comparable. These levels were in the expected physiological range for nonfasting men and women (5,11). Leptin levels were positively correlated with BMI (women: $r = 0.56$, $P < 0.001$; men: $r = 0.47$, $P = 0.007$), waist circumference (women: $r = 0.44$, $P < 0.001$; men: $r = 0.36$, $P = 0.043$), and body fat (women $r = 0.62$, $P < 0.001$; men $r = 0.69$, $P < 0.001$), but were not related to age, ethnicity, or smoking.

Table 1 Participant characteristics

	Men (n = 32)	Women (n = 62)	P value
Age (years)	21.4 (2.14)	21.4 (2.11)	0.94
Weight (kg)	74.1 (7.7)	61.4 (9.8)	<0.001
Waist (cm)	79.2 (7.0)	69.6 (7.6)	<0.001
BMI (kg/m ²)	23.4 (2.4)	22.9 (2.9)	0.41
% Body fat (impedance)	13.8 (3.8)	25.4 (5.3)	<0.001
Systolic BP (mmHg)	123 (11)	112 (10)	<0.001
Diastolic BP (mmHg)	66 (7)	65 (9)	0.70
Heart rate (bpm)	70 (11)	72 (9)	0.28
HRV (ms)	57 (33)	57 (30)	0.93
Cardiac PEP (ms)	124 (10)	123 (8)	0.40
Plasma leptin (ng/ml)	2.26 (1.99)	35.68 (21.96)	<0.001
sq leptin	1.38 (0.62)	5.71 (1.77)	<0.001
Plasma IL-6 (pg/ml)	0.78 (0.53)	0.69 (0.36)	0.37
HbA1c	4.9 (0.26)	4.8 (0.27)	0.17
% Smokers	9.4	17.7	0.38
Ethnicity			
% Non-white	25.0	34.4	0.48

Data displayed as mean (s.d.) or percentage. sq leptin is square root transformed leptin concentration. BP, blood pressure; HbA1c, glycated hemoglobin; HRV, heart rate variability; IL-6, interleukin-6; PEP, preejection period.

Cardiovascular and inflammatory responses to psychological stress

There were no significant differences in the cardiovascular responses of men and women. Tasks induced significant increases in heart rate in all participants, which returned to baseline during recovery (Figure 1a). Heart rate increased by 11.61 bpm on average during tasks, and there was wide variation in the magnitude of this response ranging from -3.47 to 34.87 bpm. There were also significant reductions in HRV and cardiac PEP during tasks. HRV decreased during tasks by 17.66 ms on average, with responses ranging from -99.44 to +12.83 ms (Figure 1b), and cardiac PEP decreased by 7.07 ms on average during tasks, with responses ranging from -28.0 to +12.95 ms (Figure 1c). Plasma IL-6 levels increased following tasks, with a significantly larger response in women (+0.26 pg/ml, 37%) vs. men (+0.02 pg/ml, 3%), $P = 0.005$. Again, there were large individual differences in this response ranging from -0.45 to 1.72 pg/ml in women.

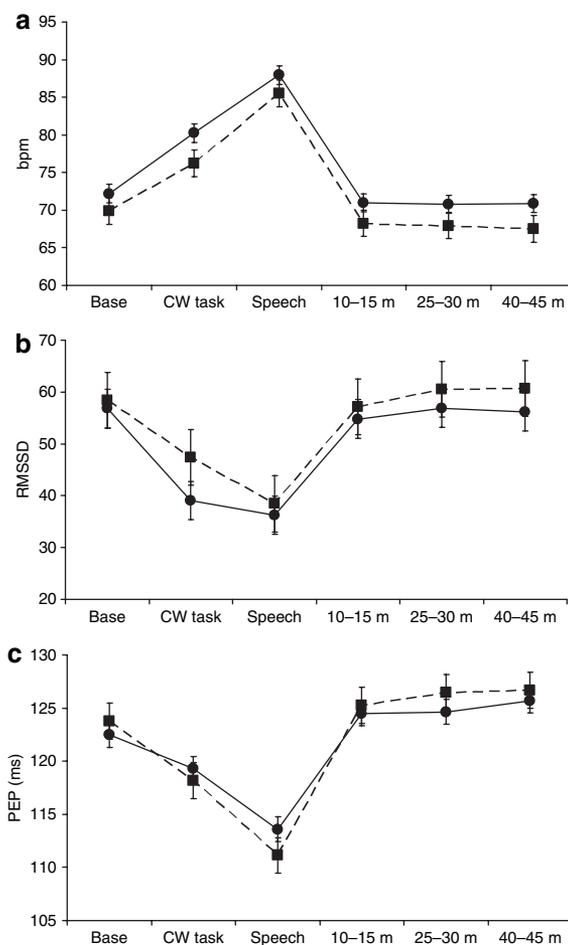


Figure 1 Cardiovascular responses to acute psychological stress in men (dotted line, squares) and women (solid line, circles). Stressful tasks induced significant increases in (a) heart rate and decreases in (b) heart rate variability and (c) cardiac preejection period (PEP) in all participants. Error bars are s.e.m. RMSSD, root mean square of successive R-R interval differences. CW task, color-word task.

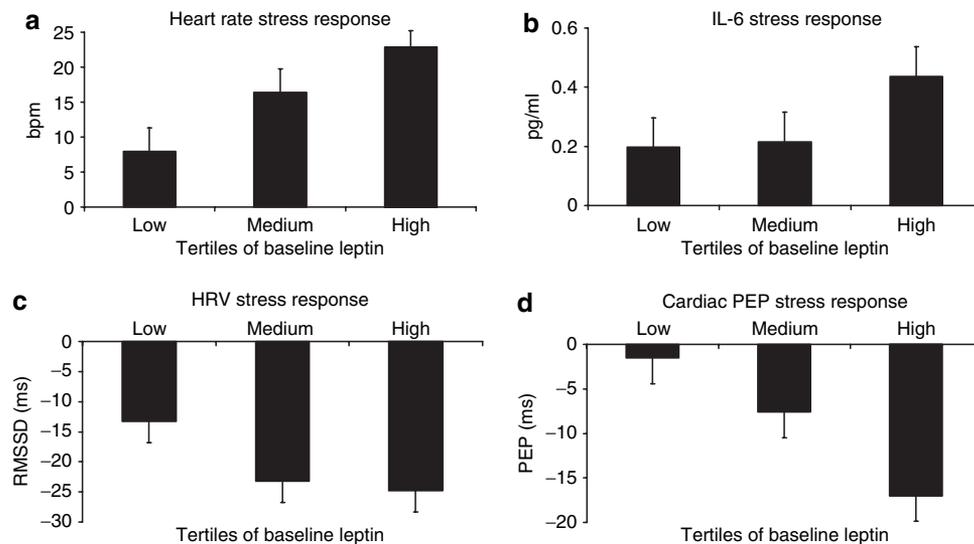


Figure 2 Mean cardiovascular and inflammatory stress responses in relation to tertiles of baseline plasma leptin in women. Baseline circulating leptin predicted stress-induced changes in (a) heart rate, (b) plasma interleukin-6 (IL-6), (c) heart rate variability (HRV), and (d) cardiac preejection period (PEP) across participants (all $P < 0.05$). Values are adjusted for age, smoking status, BMI, waist circumference, % body fat, and baseline levels of the appropriate dependent variable. Error bars are s.e.m. RMSSD, root mean square of successive R-R interval differences.

Relationship between baseline leptin and stress responses

In women, there were significant relationships between baseline plasma leptin and both cardiovascular and inflammatory stress responses, independent of age, BMI, smoking status, waist circumference, % body fat and the baseline level of the appropriate dependent variable. Women's basal leptin levels were positively associated with stress-induced increases in heart rate ($\beta = 0.53$, s.e. = 0.18, $P = 0.006$). Leptin was also significantly correlated with decreases in HRV ($\beta = -0.44$, s.e. = 0.18, $P = 0.015$) and cardiac PEP ($\beta = -0.51$, s.e. = 0.17, $P = 0.004$) during tasks, independently of covariates. In addition, there was a positive association between baseline plasma leptin and stress-induced increases in circulating IL-6 at 45-min posttask ($\beta = 0.35$, s.e. = 0.17, $P = 0.042$). In contrast, there was no relationship between baseline plasma leptin and any measure of cardiovascular or inflammatory stress response in men. The regression coefficients were 0.07 (s.e. = 0.35, $P = 0.85$) for heart rate, 0.10 (s.e. = 0.26, $P = 0.71$) for HRV, and 0.42 (s.e. = 0.26, $P = 0.11$) for cardiac PEP reactivity, and -0.05 (s.e. = 0.21, $P = 0.83$) for IL-6 responses in men. Effects in women are illustrated in [Figure 2a–d](#), showing mean levels of cardiovascular and inflammatory stress responses in relation to tertiles of baseline plasma leptin. It can be seen that women with elevated plasma leptin had greater sympathetic cardiovascular and inflammatory responses to stress, coupled with parasympathetic withdrawal. For example, heart rate responses were 187% larger among women in the highest vs. lowest tertile of baseline leptin. Reductions in HRV and cardiac PEP during tasks were 86% and >11-fold greater in women in the highest vs. lowest tertile of baseline leptin, respectively. Similarly, proinflammatory IL-6 responses to tasks were 2.2-fold higher in women with elevated baseline leptin.

DISCUSSION

Here we show that basal circulating leptin levels are an independent predictor of cardiovascular sympathetic activation and parasympathetic withdrawal following exposure to acute psychological stress in young women. These findings are in agreement with previous studies in animals and humans supporting a regulatory effect of leptin on cardiovascular autonomic function (6,7,9–13). The effects of leptin on the SNS are thought to be mainly driven by a central mechanism through binding to receptors on the hypothalamus. The long form of the leptin receptor (OB-Rb), which appears to mediate most of the biological effects of leptin, is expressed in several hypothalamic nuclei with highest expression in the arcuate nucleus. In rodents, intra-arcuate nucleus injection of leptin increases sympathetic nerve outflow to thermogenic and cardiovascular tissues as well as increasing arterial pressure (25). Conversely, the thermogenic sympathetic nerve activity response to leptin is blocked by lesions to the arcuate nucleus (25). Within the arcuate nucleus, OB-Rb is expressed in neurons containing peptides involved in feeding behavior including proopiomelanocortin. Leptin stimulates proopiomelanocortin neurons to release α -melanocyte-stimulating hormone that activates melanocortin-4 receptors, and central pharmacological blockade or genetic ablation of these receptors abolishes the hypertensive effects of chronic leptin infusion in rodents (26).

In addition to these centrally driven effects, there is emerging evidence that leptin can exert direct, nonneural effects on cardiovascular function. Leptin receptors or their mRNA have been detected in the heart and endothelium of blood vessels as well as several other peripheral organs including adipose tissue, kidney, liver, spleen, pancreas, and testes of animals (6). Furthermore, a strong independent association between circulating leptin and heart rate was observed in heart transplant patients with sympathetic denervation, supporting a direct

effect of leptin on heart rate conceivably through cardiac leptin receptors (27).

We also observed a significant correlation between basal circulating leptin levels and IL-6 responses to stress in women. In agreement with these findings, a recent investigation in 3,640 British nondiabetic men found that elevated plasma leptin was significantly correlated with plasma IL-6 levels, independent of adiposity (18), and smaller cross-sectional studies have demonstrated a positive association between circulating leptin and IL-6 in obese women (19). Notably, leptin receptors are expressed on macrophages and T-lymphocytes, and leptin stimulates the production of IL-6 and other proinflammatory cytokines by these cells (28).

The lack of correlation between leptin levels and cardiovascular or inflammatory stress responses in men is notable. Women are generally more "adipose" than men, with greater total body fat and significantly higher circulating leptin levels, and these factors could potentially contribute to the heightened association between leptin and stress responses in women (5). Importantly, associations between plasma leptin and HRV, cardiac PEP and IL-6 responses in men were marginally in the reverse direction on average to those predicted, suggesting that the observed lack of relationship between these factors was not simply due to a smaller number of male participants. Furthermore, although the standard deviation of leptin levels was lower in men than women, the coefficient of variation was actually greater (0.87 vs. 0.62), indicating that the lack of effects in men was not due to a restricted range of leptin values. Supporting our findings, a recent study of 130 healthy young men and women of equal numbers and comparable age, also found a significant correlation between leptin levels and sympathetic autonomic activity (indexed by heart rate and HRV) in women, but not in men (29).

Heightened and/or prolonged cardiovascular reactivity to acute laboratory stress is predictive of future hypertension in initially normotensive samples as well as an increased risk of future cardiac events in patients with documented cardiovascular disease (17,30). There are less data linking acute inflammatory responses with cardiovascular risk. However, a longitudinal study of 153 men and women from the Whitehall II cohort found that IL-6 responses to acute laboratory stress independently predicted elevations in ambulatory systolic BP over a 3-year period (31). Accordingly, IL-6 stimulates a number of mechanisms involved in hypertensive pathogenesis, including smooth muscle cell proliferation, expression of endothelial cellular adhesion molecules and platelet aggregation (32). Together, these findings suggest that acute cardiovascular and inflammatory responses to stress may become clinically relevant if repeated on a long-term basis and promote the development of hypertension and coronary artery disease.

This investigation was carried out in a healthy sample of young, predominately White men and women, and results may not generalize to other groups. Although our results support an association between leptin and stress-induced cardiovascular and inflammatory responses, the cross-sectional nature of the study prevents conclusions about the causal direction of this

association. We did not include direct measures of sympathetic activity such as catecholamines in our study, and it should be noted that heart rate and HRV are regulated by both parasympathetic vagal withdrawal as well as increases in sympathetic nerve activity. However, cardiac PEP is a strong indicator of sympathetic nerve activation (24). Furthermore, a number of previous reports in humans and animals also provide evidence for a sympatho-activating effect of leptin (6,7,9–13). Although our results suggest that the lack of relationship between leptin and stress responses in men was not due to a smaller number of male participants, further studies are required to confirm the observed gender differences in the relationship between leptin and stress reactivity. Cytokine analyses were conducted on nonfasting plasma samples, and there is some evidence that ingestion of a high carbohydrate or high-fat meal alters circulating leptin levels in animals (33). However, most human studies do not support an acute effect of food intake on circulating leptin at least up to 3 h following a meal, showing only a long-term dietary effect of chronic overfeeding (33). Furthermore, acute food ingestion has not been shown to influence cardiovascular reactivity to laboratory stressors (34). Breathing pattern is an important determinant of HRV, but was not measured in this study. We also did not control for menstrual phase during testing. However, differences in psychophysiological stress reactivity across the menstrual cycle have been inconsistent (35). Lastly, recent evidence has highlighted a role for the soluble form of the leptin receptor (sOB-R), in regulating leptin activity (36). Because human obesity is characterized by low circulating levels of sOB-R together with elevated leptin, the potential contribution of sOB-R to the relationship between adiposity, leptin and stress reactivity warrants further investigation.

In conclusion, our results suggest that circulating leptin is an independent predictor of sympathetic cardiovascular activity, parasympathetic withdrawal, and inflammatory responses to psychological stress in women. Because hyperleptinemia is independently associated with hypertension, the metabolic syndrome, and cardiovascular disease risk (6), the adverse health effects of stress in women may be partly mediated through leptin. Future studies should investigate the longitudinal relationship between circulating leptin/sOB-R, acute stress responses, and cardiovascular health in humans.

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DISCLOSURE

The authors declared no conflict of interest.

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