Research report

Associations of ghrelin with eating behaviors, stress, metabolic factors, and telomere length among overweight and obese women: Preliminary evidence of attenuated ghrelin effects in obesity?

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ABSTRACT

Ghrelin regulates homeostatic food intake, hedonic eating, and is a mediator in the stress response. In addition, ghrelin has metabolic, cardiovascular, and anti-aging effects. This cross-sectional study examined associations between total plasma ghrelin, caloric intake based on 3 day diet diaries, hedonic eating attitudes, stress-related and metabolic factors, and leukocyte telomere length in overweight ($n = 25$) and obese women ($n = 22$). We hypothesized associations between total plasma ghrelin and eating behaviors, stress, metabolic, cardiovascular, and cell aging factors among overweight women, but not among obese women due to lower circulating ghrelin levels and/or central resistance to ghrelin. Confirming previous studies demonstrating lowered plasma ghrelin in obesity, ghrelin levels were lower in the obese compared with overweight women. Among the overweight, ghrelin was positively correlated with caloric intake, giving in to cravings for highly palatable foods, and a flatter diurnal cortisol slope across 3 days. These relationships were non-significant among the obese group. Among overweight women, ghrelin was negatively correlated with insulin resistance, systolic blood pressure, and heart rate, and positively correlated with telomere length. Among the obese subjects, plasma ghrelin concentrations were negatively correlated with insulin resistance, but were not significantly correlated with blood pressure, heart rate or telomere length. Total plasma ghrelin and its associations with food intake, hedonic eating, and stress are decreased in obesity, providing evidence consistent with the theory that central resistance to ghrelin develops in obesity and ghrelin's function in appetite regulation may have evolved to prevent starvation in food scarcity rather than cope with modern food excess. Furthermore, ghrelin is associated with metabolic and cardiovascular health, and may have anti-aging effects, but these effects may be attenuated in obesity.

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Introduction

Ghrelin, the only known appetite-stimulating hormone in humans, is a 28 amino acid protein produced principally in the stomach. Ghrelin and its receptor, growth hormone secretagogue receptor (GHS-R), are found extensively throughout the body, indicating widespread central and peripheral functions (Gnanapavan et al., 2002; Korbonits, Goldstone, Gueorguiev, & Grossman, 2004; Muccioli, Baragli, Granata, Papotti, & Ghigo, 2007). When energy supply is low, ghrelin is secreted from gut mucosa and acts centrally by signaling to the hypothalamic arcuate nucleus and also stimulates the vagal afferent nerves (Date & Kangawa, 2012) to increase appetite and food intake. After food ingestion, plasma levels decrease (Couce et al., 2006).

In addition to the regulation of homeostatic food intake ghrelin appears to be involved in hedonic eating, and may be necessary for the experience of food-induced reward (Diz-Chaves, 2011; King, Isaacs, O'Farrell, & Abizaid, 2011; Kirsz & Zieba, 2011; Skibicka, Hansson, Alvarez-Crespo, Friberg, & Dickson, 2011). There is evidence that ghrelin increases preference for sweet taste (Disse et al., 2010; Malik, McGlone, Bedrossian, & Dagher, 2008). Moreover, ghrelin was found to *increase*, rather than decrease, in response to palatable food intake under conditions of satiety, suggesting that ghrelin's central signaling may drive hedonic food

consumption in the absence of caloric need (Monteleone et al., 2013).

Furthermore, accumulating evidence suggests that ghrelin signaling is important in the neurological response to stressors (Asakawa et al., 2001; Chuang et al., 2011; Raspopow, Abizaid, Matheson, & Anisman, 2010; Rouach et al., 2007) and stress increases ghrelin in humans (Harrold, Dovey, Blundell, & Halford, 2012; Lowe & Butryn, 2007). As stress induces eating and the motivation to eat comfort foods (Adam & Epel, 2007; Dallman, 2010), increased ghrelin may mediate the motivation to eat under stress.

Studies of diet-induced obese mice have demonstrated hypothalamic resistance to ghrelin, in which ghrelin no longer stimulates activation of neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurons, which trigger hunger (Briggs, Enriori, Lemus, Cowley, & Andrews, 2010; Briggs et al., 2013; Finger, Dinan, & Cryan, 2012). Diet-induced obesity in mice suppresses the neuroendocrine ghrelin system by decreasing mRNA of ghrelin and the enzyme which converts ghrelin to its active acylated form (ghrelin o-acyltransferase) in the stomach, expression of GHS-R in the hypothalamus, and acylated and total plasma ghrelin (Briggs et al., 2010). In humans, plasma ghrelin levels have been found to be substantially lower among obese compared to lean adults (Druce et al., 2005; McLaughlin, Abbasi, Lamendola, Frayo, & Cummings, 2004; Ozkan et al., 2009; Tschop et al., 2001). These findings have led to the speculation that obesity may induce central ghrelin resistance in humans, and relationships between ghrelin and eating behavior observed in lean adults may not apply to obese populations (Andrews, 2011). However, little research has examined the central resistance theory in obese humans. Furthermore, it is unclear whether evidence of central ghrelin resistance would be observed in an overweight population, presumably where there is also decreased plasma ghrelin, but perhaps not as low as levels found in obese populations (Cummings, 2006; Sumithran et al., 2011; Tschop et al., 2001).

In terms of other actions of ghrelin, ghrelin has been associated with increased plasma glucose levels, decreased insulin and insulin resistance, and with lower blood pressure and heart rate (Broglio et al., 2001; Eizadi, Afsharmand, Behbudi, & Sohaily, 2011; Freeman, Carmo, Adi, & da Silva, 2013; Garcia & Korbonits, 2006; Okumura et al., 2002; Tong et al., 2010; Verhulst & Depoortere, 2012). Ghrelin may also have anti-aging effects as it reduces inflammation (Dixit et al., 2004) and regulates growth hormone secretion (Veldhuis & Keenan, 2012), which controls secretion of insulin-like growth factor-1 (IGF-1). Lower levels of IGF-1 are related to shorter telomere length, which is a marker of cell aging (Barbieri et al., 2009; Kaplan et al., 2009; Moverare-Skrtic et al., 2009), and which has been linked to diabetes risk factors (Demissie et al., 2006; Gardner et al., 2005) and earlier mortality (Blackburn, Greider, & Szostak, 2006; Cawthon, Smith, O'Brien, Sivatchenko, & Kerber, 2003; Epel et al., 2009; Fitzpatrick et al., 2011). Epidemiological studies have shown that telomere length is moderately correlated with chronological age (for a review, see Sanders & Newman, 2013). Telomere length is thought to represent biological aging of the cell in that the longer the telomere length, the greater the cell's ability to keep dividing, conversely, the shorter the telomere length, the greater the cell's replicative senescence (Blackburn, 2000). As peripherally circulating ghrelin appears to exert some beneficial metabolic, cardiovascular and anti-aging effects (Aoki et al., 2013; Garcia & Korbonits, 2006; Granado, Priego, Martin, Villanua, & Lopez-Calderon, 2005), it is possible that the lowered circulating levels found in obesity may negatively impact ghrelin's favorable physiological effects.

Indeed, there are many putative roles for ghrelin beyond homeostatic appetite control, and research is needed to describe and understand the multiplicity of ghrelin's functions and any differential functions of ghrelin among overweight and obese adults.

In this preliminary cross-sectional analysis, we examined associations of ghrelin with caloric intake, hedonic eating, psychological and physiological indicators of stress, metabolic and aging-related factors among overweight and obese women. First, we hypothesized that circulating plasma ghrelin levels would be lower among obese compared to overweight women. Second, we hypothesized that ghrelin may be positively related to food intake, hedonic eating, and stress among overweight women, but that such correlations would be attenuated among obese women. We also aimed to determine whether ghrelin's previously established associations with glucose and insulin levels, blood pressure, and heart rate would be observed among the overweight and obese samples. Finally, we explored the relationship in both groups between ghrelin and leukocyte telomere length, an association that has not yet been examined in humans or animals to our knowledge.

Methods

This study is a cross-sectional, secondary analysis of baseline data collected from a randomized controlled pilot study of a mindfulness intervention to reduce stress eating (Daubenmier et al., 2011). As previously reported in the parent study, overweight and obese women were recruited from the San Francisco Bay Area community using flyers and local media. Exclusion criteria included: weight over 300 lbs, diabetes, taking medication that could affect weight loss, taking pain steroids or antipsychotic medication, post-menopausal, history of bilateral oophorectomy, total hysterectomy, polycystic ovary syndrome, active endocrine disorder, pregnancy, less than 1 year postpartum or breastfeeding, current eating disorder, alcohol addiction, drug addiction, positive urine test for diabetes and opiate use, and English illiteracy. A total of 322 women were screened, and 47 women with Body Mass Index (BMI) of 25–40 were enrolled into the study and completed 2 baseline assessments by staff at the UCSF General Clinical Research Center (GCRC) (Daubenmier et al., 2011). This study was performed at the University of California, San Francisco (UCSF) with approval from the institutional review board.

Biological measures

At the first baseline visit, a digital scale (Wheelchair Scale 6002, Scale-Tronix, Carol Stream, IL) was used to measure weight in kg to the nearest 0.10 kg and a standard stadiometer (Perspective Enterprises, Portage, Mich, USA) was used to measure height to the nearest 1/8 in. to calculate BMI. Blood pressure and heart rate were also measured (CritikonDinamap 1846SX Non Invasive Vital Signs Monitor, GE Healthcare, Milwaukee, WI). To assess blood pressure, participants rested for 5 min and three measurements were taken, each 1 min apart. The three measurements were averaged to determine systolic and diastolic blood pressure values.

At the second baseline visit, nurses confirmed with participants that they completed a 12 h fast the night before. Morning blood plasma samples were taken from an indwelling forearm catheter for total plasma ghrelin levels, insulin, glucose, and telomere length. Blood samples were drawn into tubes on ice containing ethylenediaminetetraacetic acid (EDTA), an anticoagulant, and kept on ice at all times. The tubes were centrifuged for 10 min at 3000 g at 4 °C, transferred to aliquot vials, and frozen at −70 °C until assaying.

Total plasma ghrelin was measured without an extraction step using a commercial RIA (Phoenix Peptide, Phoenix, AZ), as described previously (Cummings, Clement, et al., 2002; Cummings et al., 2001). Insulin was assayed with a radioimmunoassay kit using an I125-Iodinated insulin tracer, anti-Human Insulin Specific antibody, and human insulin standards from Linco Research, Inc.

(St. Charles, MO). The intra- and inter-assay variations are 5.8% and 10.2% respectively. Glucose was measured enzymatically (glucose oxidase) with an automated YSI 2300 Analyzer from YSI Life Sciences (Yellow Spring, Ohio). Instrument precision is within 2%. Insulin resistance was measured by homeostatic model assessment (HOMA) from values obtained from plasma glucose and insulin assays (Wallace, Levy, & Matthews, 2004).

Salivary cortisol was measured by participants at home. Participants were instructed on how to collect saliva samples by salivating into a straw in 2 ml "SaliCaps" tubes. They were instructed to collect the first sample before getting out of bed, and not to eat, drink or brush their teeth or engage in vigorous activity between the morning samples or for 20 min before the evening sample. Participants collected saliva samples upon awakening, 30 min later, and prior to bedtime. Samples for the cortisol awakening response (CAR) were collected each day over 4 days, and samples for the cortisol slope were collected each day over 3 days. The CAR was calculated by subtracting the 30-min post awakening sample from the waking sample. Higher CARs are related to greater perceptions of life stress (Chida & Steptoe, 2009). Cortisol slope was calculated by subtracting the bedtime value from the morning value such that higher values indicated steeper slopes. Flatter cortisol slopes are associated with threatening, traumatic, and uncontrollable experiences of stress (Schulkin, 2004). Values were averaged over the days collected. Hormone analysis was performed at Dresden Lab Service, overseen by Dr. Clemens Kirschbaum, at the Dresden University of Technology (Germany) using a commercial chemiluminescence immunoassay (CLIA, IBL, Hamburg, Germany). Values greater than 100 mg/dl were excluded as it is probable that these values are physiologically abnormal, possibly due to blood contamination or a medical or assay issue. They are also statistical outliers.

Mean telomere length was measured quantitatively in peripheral blood nuclear cells. Telomere length was measured from DNA by a quantitative polymerase chain reaction (PCR) assay that determines the relative ratio of telomere repeat copy number to single-copy gene copy number (T/S ratio) in experimental samples as compared with a reference DNA sample, as described previously (Kiecolt-Glaser et al., 2013). All PCRs were carried out on a Roche Lightcycler 480 real-time PCR machine with 384-tube capacity (Roche Diagnostics Corporation, Indianapolis, IN) (Lin et al., 2010).

Self-report measures

Three day diet diaries

Participants were given paper forms to complete food diaries over a 3 day period. As the participants consumed food, they were instructed to record the types of food they ate and portion sizes. The diaries were used to assess total daily caloric intake, and percentages of dietary calories from carbohydrates, fats, proteins, and sugar. The Food Processor SQL software was used for nutritional analysis.

Hedonic eating attitudes

Participants completed on-line questionnaires at home. Hedonic eating attitudes were measured using the Food Craving Inventory, which measures specific cravings and giving in to cravings for foods high in fat, sugar, carbohydrates, and high fat fast-food over the previous month. A craving was defined as "an intense desire to consume a particular food (or food type) that is difficult to resist," and the subscales ask participants to rate the frequency of cravings on a 5-point Likert scale (1 = never, 2 = rarely, 3 = sometimes, 4 = often, and 5 = always/almost every day) (White, Whisenhunt, Williamson, Greenway, & Netemeyer, 2002). Examples of foods in the high fat category included fried fish, fried chicken, hot dog; the sweet category included brownies, cookies, cakes; and the fast food fats category included hamburgers, French fries, pizza. To provide a robust measure of highly palatable food cravings, two composite scores were created. For the composite score of craving highly palatable foods, we averaged the scores for high fat, sweet, and fast-food fats. To measure "giving in" to cravings for highly palatable foods, we averaged the scores for giving in to cravings for high fat, sweet, and fast food fats. Cronbach's alpha for these composite measures of the Food Craving Inventory in this sample was 0.86 for craving highly palatable foods, and 0.91 for giving in to cravings.

Psychological stress

Psychological stress was measured using the 10-item version of the Perceived Stress Scale. This measure utilizes a 5-point Likert scale to evaluate the degree to which individuals consider their lives to be stressful over the previous month (from 0 = never, to 4 = very often). Scores range from 0 to 40, with higher scores associated with higher perceived stress (Cohen & Janicki-Deverts, 2012; Cohen, Kamarck, & Mermelstein, 1983). Cronbach's alpha for the Perceived Stress Scale in this study was 0.84.

Emotional eating

Emotional eating is an individual's trait behavior of eating in response to negative emotions. Emotional eating was measured with the Emotional Eating subscale of the Dutch Eating Behavior Questionnaire. The scale asks participants to rate their desire to eat in response to clearly labeled and diffuse emotions, such as boredom and anger using a 5-point Likert scale (ranging from 1 = never to 5 = very often). It has been widely used to assess emotional eating in a variety of populations including obese women (Pinaquy, Chabrol, Simon, Louvet, & Barbe, 2003; van Strien, Frijters, Bergers, & Defares, 1986). Cronbach's alpha for the Emotional Eating subscale in this sample was 0.78.

Statistical analysis

Distributions and descriptive statistics of all variables were reviewed, including means and standard deviations for continuous variables. Non-normal, skewed distributions were natural log transformed. Student's *t*-tests and chi-square tests were performed to compare baseline characteristics between the overweight and obese groups. The associations between ghrelin and the measured variables were examined using Pearson correlations. One overweight participant, and three obese participants were taking anti-hypertensive medications at the time of the study, and their data were excluded from the correlations of plasma ghrelin with blood pressure. All data were analyzed using Stata 12.0 (College Station, TX, USA).

Results

Sample characteristics

The sample characteristics are described in detail in the report on the mindfulness intervention for stress eating (Daubenmier et al., 2011). Out of 47 participants measured at baseline, 25 women were overweight, and 22 obese. As described previously, the sample identified as 62% white, 15% Hispanic/Latino, 15% Asian/Pacific Islander, and 9% other. The groups were similar in regards to white/non-white status by BMI group (*p* = .34). Participants were found to have higher mean levels of perceived stress, compared to a representative U.S. population sample, and higher mean levels of emotional eating compared to a representative Dutch sample (Daubenmier et al., 2011).

The characteristics of the overweight and obese women are described in Table 1. As expected, total fasting plasma ghrelin was

lower in the obese compared to the overweight women (see Figs. 1 and 2 for scatterplots). There were no statistically significant differences in total daily calorie intake between the groups. However, there were differences in percentage of caloric intake from fat and carbohydrates between the two groups, with the obese women consuming a greater percentage of calories from fat, and lower percentage from carbohydrates compared to the overweight women. No statistically significant differences between groups were found in perceived stress, emotional eating, or hedonic eating attitudes. Among the metabolic factors, the obese women had higher mean insulin levels, and higher mean insulin resistance measured by HOMA compared to the overweight women. The groups were similar in regards to blood pressure and heart rate. There was no evidence of a significant difference in telomere length between the groups, nor after controlling for age ($p = .21$).
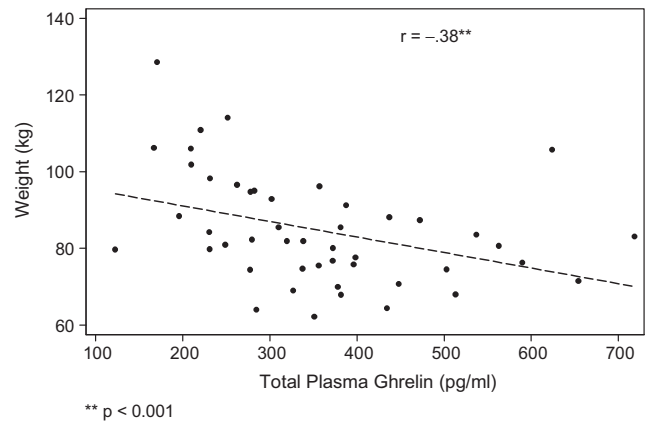

** p < 0.001

Fig. 1. Scatterplot of the relation between weight (kg) and plasma ghrelin.

Correlations with plasma ghrelin and other variables

Correlations of total plasma ghrelin with food intake, hedonic eating attitudes, stress related factors, metabolic factors, and telomere length by weight status are shown in Table 2 and Figs. 3–9. Among the overweight, ghrelin was positively associated with average daily caloric intake and negatively correlated with percentage of calories from protein. Ghrelin appeared to be positively related to percentage of calories from sugar, but this did not reach statistical significance ($p = .058$). These correlations were non-significant among the obese group. Ghrelin was not significantly related to percentage of calories from fat or carbohydrate in either group.

In terms of hedonic eating attitudes, ghrelin was positively associated with giving into palatable foods in the overweight, but not in the obese group. Ghrelin was not associated with cravings for palatable foods in either group. Correlations of plasma ghrelin with perceived stress, emotional eating, and the CAR were negligible in both groups. However, ghrelin was significantly negatively

related to average cortisol slope in the overweight group, but the relationship was non-significant in the obese group.

Ghrelin was not related to glucose, but was significantly negatively correlated with insulin and insulin resistance in both the overweight and obese groups. Excluding data from four participants who were taking anti-hypertensive medications, ghrelin was negatively correlated with systolic blood pressure in both groups, although the correlation did not reach statistical significance in the obese group ($p = .06$). There was some evidence to indicate that ghrelin was negatively correlated with diastolic blood pressure in the overweight group, although not statistically significant ($p = .07$), but we did not find any evidence of a strong correlation in the obese group.

Age was not significantly correlated with telomere length among the overweight ($r = .08$, $p = .70$), although it was among the obese group ($r = -.53$, $p < .05$). Telomere length was significantly positively correlated with ghrelin bivariately among the overweight women, but not among the obese group, nor after controlling for age

Table 1
Baseline characteristics of overweight and obese women.

|	n	Overweight Mean (SD)	n	Obese Mean (SD)	p value
Age (years)	25	41.76 (7.2)	22	39.9 (7.5)	0.39
Weight (kg)	25	75.17 (7.1)	22	95.7 (12.6)	<0.01
Total plasma ghrelin (pg/ml)	25	409.27 (128.9)	22	294.45 (116.2)	<0.01
Food intake – Diet diaries					
Average daily calories	22	2086.59 (542.6)	20	2127.48 (412.0)	0.78
% of calories from fat	22	32.21 (4.4)	20	35.67 (6.4)	0.05
% of calories from protein	22	17.78 (6.2)	20	17.62 (4.0)	0.92
% of calories from carbohydrates	22	50.41 (5.8)	20	45.53 (7.2)	0.02
% of calories from sugar	22	17.32 (6.2)	20	15.48 (4.3)	0.27
Hedonic eating attitudes					
Craving palatable foods	24	51.67 (10.0)	22	48.00 (12.6)	0.28
Giving into palatable foods	23	43.95 (15.3)	21	42.76 (15.7)	0.80
Stress-related factors					
Perceived stress	25	19.56 (6.6)	22	18.45 (5.0)	0.69
Emotional eating	24	3.29 (0.7)	22	3.56 (0.8)	0.23
Cortisol slope (nmol/L)	19	15.23 (6.6)	23	13.83 (4.1)	0.41
Cortisol awakening response (nmol/L)	23	7.06 (7.8)	22	6.90 (8.1)	0.95
Metabolic, cardiovascular, and cell aging factors					
Glucose (mg/dl)	25	91.94 (5.5)	22	92.58 (9.7)	0.79
Insulin (μU/ml)	25	10.86 (5.7)	22	16.5 (9.6)	0.02
Insulin resistance (HOMA)	25	2.48 (1.3)	22	3.86 (2.6)	0.03
Systolic blood pressure (mmHg)	25	117.27 (14.0)	22	123.3 (11.9)	0.12
Diastolic blood pressure (mmHg)	25	72.21 (11.1)	22	71.70 (6.4)	0.84
Heart rate (bpm)	25	73.36 (9.8)	22	74.14 (13.9)	0.83
Telomere length (T/S ratio)	25	1.11 (0.16)	20	1.08 (0.18)	0.28
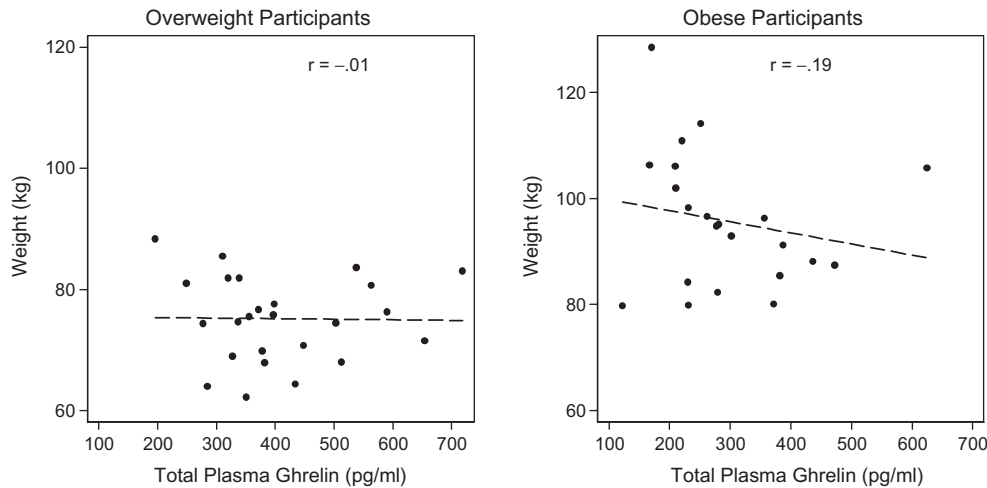
Fig. 2. Scatterplots of the relation between weight (kg) and plasma ghrelin by BMI group.

Table 2
Pearson correlations of ghrelin with eating, stress, and metabolic factors, and telomere length by group.

	Overweight		Obese	
	n	*r*	*n*	*r*
Three day diet diaries				
Average daily calories	22	.51**	20	−.28
% of calories from fat	22	.14	20	−.27
% of calories from protein	22	−.43*	20	.19
% of calories from carbohydrate	22	.25	20	.13
% of calories from sugar	22	.41^	20	.08
Hedonic eating attitudes				
Craving palatable foods	24	.24	22	.10
Giving into palatable foods	23	.44*	21	.05
Stress-related factors				
Perceived stress	25	.12	22	.05
Emotional eating	24	.25	22	.29
Cortisol awakening response (nmol/L)	23	−.01	22	.11
Cortisol Slope (nmol/L)	19	−.48*	23	.27
Metabolic, cardiovascular, and cell aging factors				
Glucose (mg/dl)	25	−.16	22	.02
Insulin (μU/ml)	25	−.57**	22	−.43*
Insulin resistance (HOMA) (ln)	25	−.66**	22	−.49*
Systolic blood pressure (mmHg)[a]	24	−.46*	19	−.44^
Diastolic blood pressure (mmHg)[a]	24	−.38^	19	−.25
Heart rate (bpm)	25	−.40*	22	.00
Telomere length (T/S ratio)	25	.41*	20	−.28

[a] Excluding participants taking anti-hypertensive medication.
^ $p < .10$.
* $p < .05$.
** $p < .01$.

(partial $r = −.04$, $p = .88$). In order to further understand the association between ghrelin and telomere length among the overweight group, we conducted exploratory analyses correlating telomere length with dietary (total daily caloric intake, percentages of dietary calories from carbohydrates, fats, proteins, and sugar) and metabolic (glucose, insulin, cortisol, insulin resistance, blood pressure and heart rate) factors to identify potential dietary and biological mediators. We found no significant correlations with telomere length except for percentage of calories from protein, which was negatively related to telomere length ($r = −.59$, $p < .05$).

Discussion

Our results are consistent with those of previous studies showing that plasma ghrelin decreases with weight gain, as the obese women had significantly lower levels of ghrelin than the overweight women. Further research is needed to elucidate the relationship between body weight and circulating ghrelin to address whether weight gain is a necessary factor for depleting circulating ghrelin levels, or if it is a marker for other underlying metabolic changes affecting total plasma ghrelin levels.

We found positive associations between ghrelin and eating behaviors in the overweight, consistent with findings from studies of normal weight adults and animals (Dickson et al., 2011; Disse et al., 2010; Malik et al., 2008; Skibicka et al., 2011). Specifically, we found that ghrelin was positively associated with daily caloric intake. There was also some evidence in support of ghrelin's role in hedonic eating as overweight women reported giving in more to eating palatable foods and ghrelin was also positively associated with sugar intake, although the correlation did not quite reach statistical significance ($p = .058$). These findings provide support for the notion that ghrelin may promote hedonic eating in the overweight, as described in prior studies of lean adults and animals (Chuang et al., 2011; Monteleone et al., 2012; Perello & Zigman, 2012; Schellekens, Dinan, & Cryan, 2013). However, this pattern of results was not observed in the obese group. Correlations of ghrelin with food intake and hedonic eating attitudes were weak or tended to be negatively correlated.

One explanation for the differential pattern of findings among the overweight and obese groups is that obesity produces resistance to central signaling of ghrelin in the hypothalamus (Briggs et al., 2010). It is not yet known whether resistance also occurs in parts of the brain circuitry that signal food reward, such as the ventral tegmental area. Of note, counter to the theory of central ghrelin resistance, are the results of one study in which administration of intravenous ghrelin to overweight and obese adults increased both intake and palatability of food (Druce et al., 2005). Therefore, lack of association of ghrelin with homeostatic and hedonic eating behaviors in obesity may be attributable to lowered circulating plasma ghrelin levels or central resistance to ghrelin. Future work replicating many of the paradigms examining the role of ghrelin in hedonic eating behaviors should be conducted among obese individuals.

Even though plasma ghrelin is reduced and may not impact eating behavior in obesity, ghrelin levels increase after weight loss (Cummings, Weigle, et al., 2002; Hansen et al., 2002; Sumithran et al., 2011). Researchers have suggested that the increase in circulating ghrelin, particularly after calorie-restricted weight loss, may make individuals more vulnerable to weight regain as the NPY and AgRP neurons in the hypothalamus become re-sensitized to ghrelin's signaling (Briggs et al., 2013). Further longitudinal study in
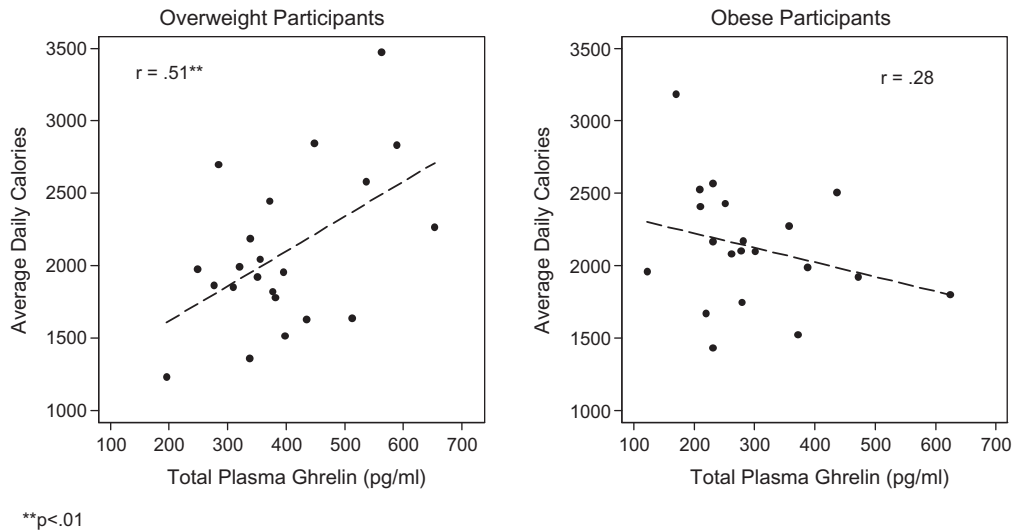
Fig. 3. Scatterplots of the relation between average daily calories and plasma ghrelin by BMI group.
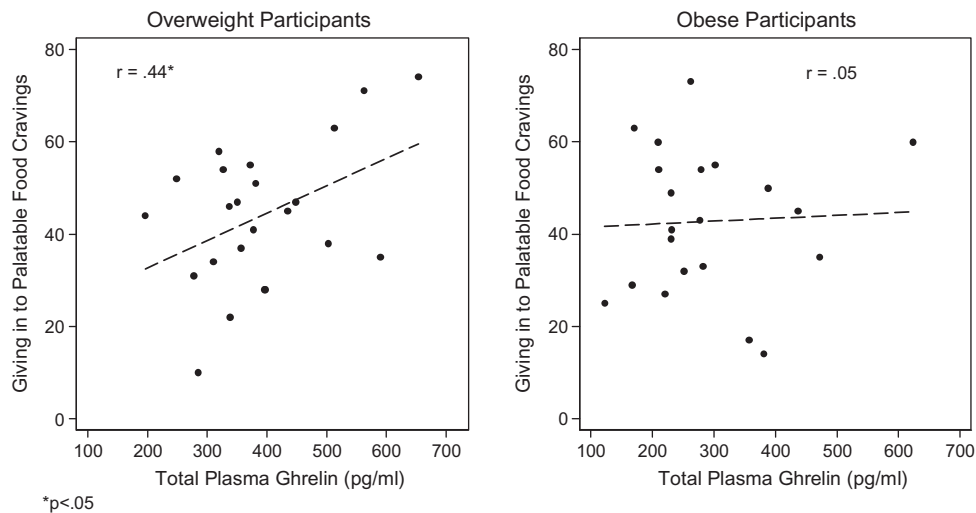
Fig. 4. Scatterplots of the relation between giving into palatable food cravings and plasma ghrelin by BMI group.

humans is needed to observe if weight loss and rebound in plasma ghrelin restores sensitivity to ghrelin signaling and if weight regain occurs as a result of ghrelin re-sensitization. It has been suggested that decreased ghrelin in obesity represents a physiological adaptation to positive energy balance – rather than having a purpose in diet-induced obesity, ghrelin's key role is to maintain homeostasis in times of negative energy balance. In other words, ghrelin's function in appetite regulation may have evolved to prevent starvation in food scarcity rather than cope with food excess (Andrews, 2011; Briggs & Andrews, 2011).

Reduced circulating ghrelin or central resistance in obesity may impair ghrelin's regulation of the HPA axis, as ghrelin stimulates corticotropin-releasing hormone (CRH) secretion in mice (Asakawa et al., 2001). Studies have also shown that ghrelin is responsive to stress and may play a mediating role in stress-related eating (Chuang et al., 2011; Rouach et al., 2007). In support, we found that a flatter diurnal cortisol slope, suggesting greater cortisol concentrations in circulation throughout the day, was associated with higher ghrelin levels among overweight women (Anders, 1982; Desir et al., 1980). However, we did not find this relationship in the

obese group. We did not find that ghrelin was related to self-reported psychological stress or emotional eating in either weight category. These null findings may be due to the restricted range of these variables as our sample reported significantly higher levels of stress and emotional eating compared to representative samples. In addition, as cortisol may be responsive to other factors aside from psychological stress and we did not observe a significant relationship between ghrelin and the cortisol awakening response (Fries, Dettenborn, & Kirschbaum, 2009), future research could examine associations with ghrelin in laboratory controlled studies of stress to better determine the relation between ghrelin and stress as a function of weight status.

While we found evidence supporting the view that ghrelin's actions may be impaired in obesity, we found evidence that some of ghrelin's metabolic and cardiovascular-related actions may remain intact while others may be impaired. Specifically, in both groups of women we found that ghrelin was negatively related to insulin resistance, as found in previous research (Amini et al., 2012; Poykko et al., 2003). As plasma ghrelin decreases in the shift
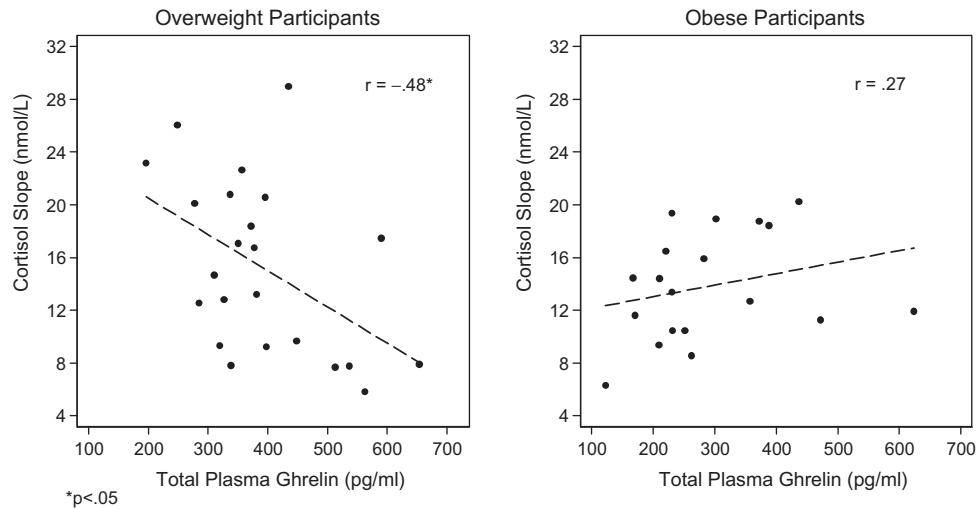
Fig. 5. Scatterplots of the relation between cortisol slope and plasma ghrelin by BMI group.
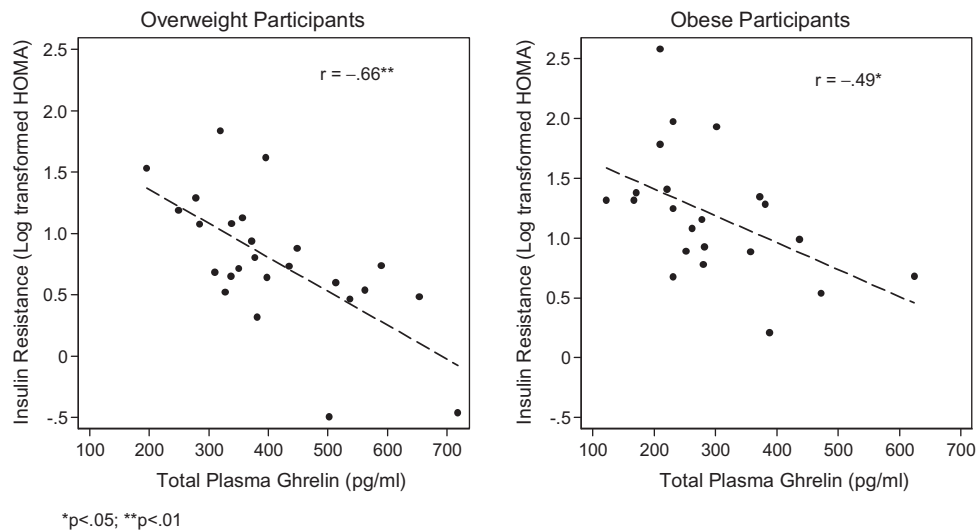

Fig. 6. Scatterplots of the relation between insulin resistance (log transformed HOMA) and plasma ghrelin by BMI group.

towards obesity, there may be increases in insulin levels and insulin resistance, which affect normal glucose homeostasis, and may increase risk for metabolic disease. Ghrelin's action in insulin resistance may be due to the effect of higher plasma acylated ghrelin in obesity. Plasma ghrelin consists of acylated (A-GHR) and non-acylated (NA-GHR). NA-GHR accounts for 80–90% of circulating ghrelin; however, in obesity the ratio of A-GHR to NA-GHR may be higher, and higher A-GHR is associated with insulin resistance (Barazzoni et al., 2007; Pacifico et al., 2009). In addition, we found ghrelin to be negatively correlated with systolic blood pressure among the overweight and negatively correlated, although not statistically significant, in the obese group. Ghrelin is associated with lower blood pressure in healthy, normal weight adults (Garcia & Korbonits, 2006; Okumura et al., 2002). The mechanism by which ghrelin lowers blood pressure is not yet fully described; there is evidence to suggest that it may be regulated through central mechanisms (Lin et al., 2004) and peripheral mechanisms by increasing diuretic action through increased renal nitric-oxide production (Aoki et al., 2013). Heart rate was also negatively correlated with ghrelin among the overweight women, but not in the obese group.

Central mechanisms may regulate heart rate as ghrelin modulates baroreflex-regulation of sympathetic vasomotor tone (Krapalis et al., 2012). It may be possible that as circulating ghrelin decreases or central ghrelin resistance increases in obesity some beneficial cardiovascular effects may be reduced and this could potentially explain the null association of ghrelin with heart rate and attenuated associations between ghrelin and blood pressure among the obese group.

Finally, growing research suggests that ghrelin may have anti-aging effects (Dixit et al., 2004; Granado et al., 2005). We explored the relationship between ghrelin and leukocyte telomere length, a marker of aging (Sanders & Newman, 2013). Longer telomeres have been related to less inflammation and higher levels of IGF-1, which, in part, are regulated by ghrelin (Barbieri et al., 2009; Dixit et al., 2004; Kaplan et al., 2009; Moverare-Skrtic et al., 2009). We found that ghrelin was positively associated with telomere length among the overweight but not among the obese women. We did not assess inflammatory markers or IGF-1 in this study so could not examine these factors as potential mechanisms. However, we found that ghrelin was negatively associated with daily percentage
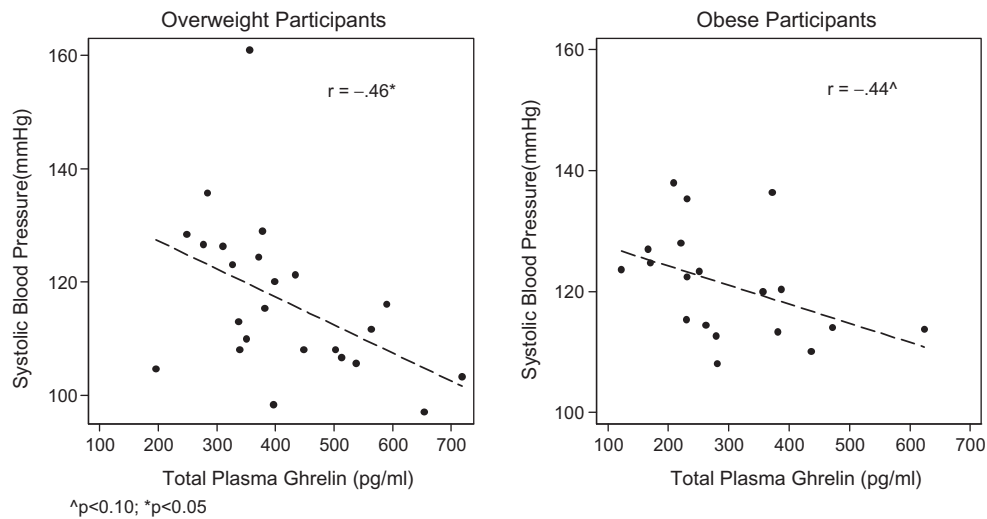
Fig. 7. Scatterplots of the relation between systolic blood pressure and plasma ghrelin by BMI group. Excluding participants taking anti-hypertensive medication.
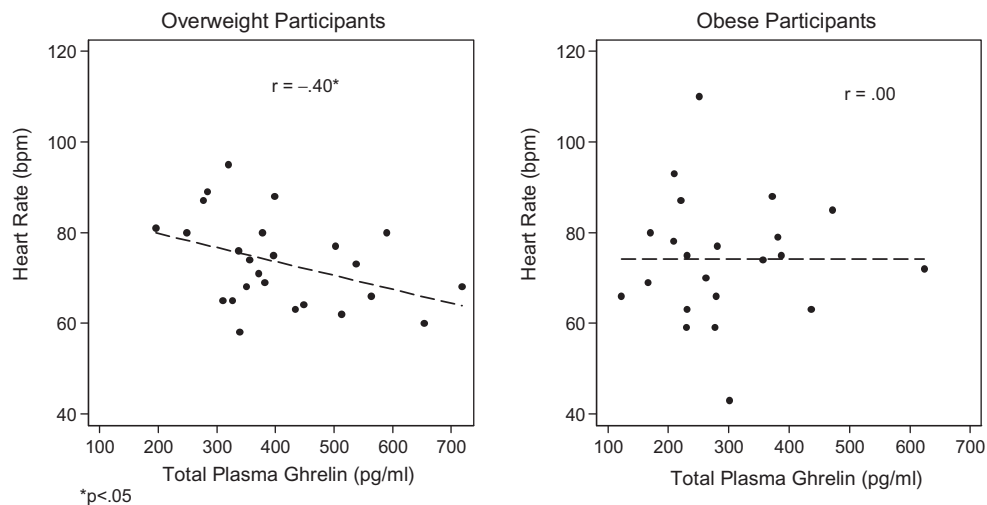

Fig. 8. Scatterplots of the relation between heart rate and plasma ghrelin by BMI group.

of calories from protein, and, in exploratory analyses, less dietary protein intake was associated with greater telomere length among the overweight women. Animal studies have shown that decreased protein intake is associated with longer telomeres (O'Callaghan et al., 2012; Tanrikulu-Kucuk & Ademoglu, 2012). Therefore, lower dietary protein intake might have a beneficial effect on telomere length. Overweight women with higher plasma ghrelin levels may eat foods higher in carbohydrates and fats, and as percent calories from macronutrients are dependent on one another, protein consumption may decrease. Alternatively, there is evidence to suggest that protein intake suppresses ghrelin levels, at least acutely (Blom et al., 2006; Bowen, Noakes, & Clifton, 2006; Brennan et al., 2012; Erdmann, Topsch, Lippl, Gussmann, & Schusdziarra, 2004; Foster-Schubert et al., 2008; Moran et al., 2005; Tannous dit El Khoury, Obeid, Azar, & Hwalla, 2006). Further research is needed to elucidate the pathways by which ghrelin may affect cellular aging mechanisms, such as telomere length, and whether these pathways are attenuated in obesity.

Limitations

Our analysis is cross-sectional and therefore we are unable to determine causality. The study sample did not include men,

therefore we do not know whether there are gender differences in the associations that we found. Our results should also be considered preliminary due to a small sample size. Further research is needed to examine and replicate the associations we described, in particular, by including normal weight adults. We measured total plasma ghrelin, but circulating ghrelin is found as acylated and non-acylated, both with different roles in promoting appetite and feeding (Adams, Greenway, & Brantley, 2011). Although total plasma ghrelin is lower in obesity, the ratio of acylated to non-acylated may increase in obesity (Pacifico et al., 2009). Future work should examine both acylated and non-acylated ghrelin in obesity.

Central ghrelin sensitivity may be affected by circulating leptin concentrations (Hewson, Tung, Connell, Tookman, & Dickson, 2002). Moreover, as ghrelin promotes feeding and leptin suppresses feeding, there is evidence to suggest that plasma ghrelin levels are negatively associated with plasma leptin levels and their actions on hypothalamic neurons may be oppositional, at least in normal weight adults and animals (Lockie & Andrews, 2013). However, diet-induced obese individuals are resistant to the effects of leptin (Mantzoros et al., 2011). Further research is needed to examine the relation between ghrelin and leptin in obesity.
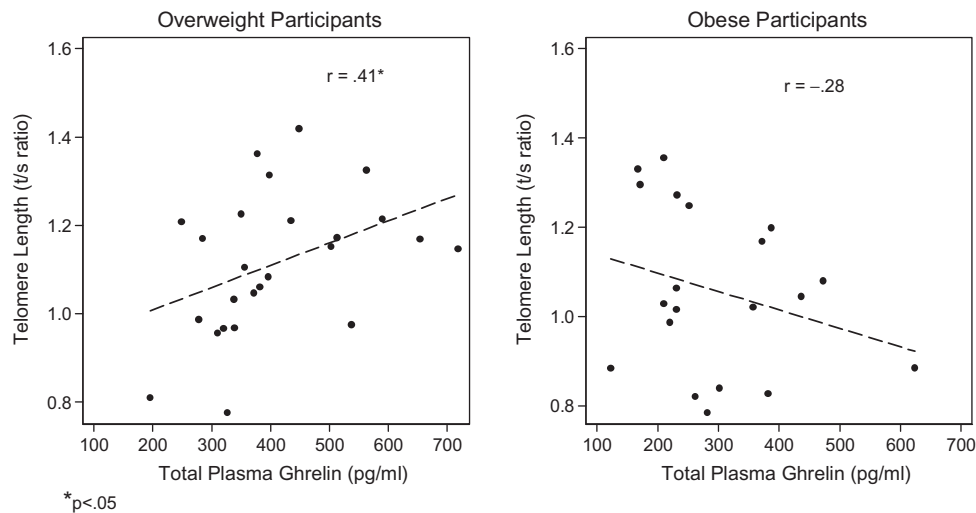
Fig. 9. Scatterplots of the relation between telomere length and plasma ghrelin by BMI group.

Some studies (Al-Massadi et al., 2010; Kellokoski et al., 2005; Matsubara et al., 2004; Shibata et al., 2004), but not all (Dafopoulos, Sourlas, Kallitsaris, Pournaras, & Messinis, 2009; Gualillo et al., 2001) have found associations with ghrelin levels and ovarian hormones, so it is possible that the findings in this study are affected by participants' menstrual cycle or use of oral contraception. Postmenopausal, and pregnant and breastfeeding women were excluded from this study, as were those with a history of polycystic ovary syndrome, oophorectomy, and hysterectomy.

Hedonic eating, diet diaries and psychological stress were measured using self-report instruments. Self-report data may introduce error as individuals may not recall information with accuracy, give socially desirable responses, or choose not to respond. Hedonic eating and psychological stress measures were collected on-line when participants were at home. We did not record or control for the time of day or other situational factors that may have influenced participant self-reports.

Conclusion

Our results indicate a complex picture of the potential health impact of circulating ghrelin concentrations in overweight and obesity. Ghrelin in overweight people may contribute to hedonic eating and eventual weight gain. However, lowered circulating ghrelin and/or central resistance to ghrelin signaling in obesity may reduce the impact of ghrelin on homeostatic energy regulation and hedonic eating. Ghrelin may have beneficial metabolic, cardiovascular and anti-aging effects and these effects may be attenuated in obesity. Future work is needed to examine the role of ghrelin in obesity, especially as regards eating behavior.

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