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## Cortisol and ghrelin concentrations following a cold pressor stress test in overweight individuals with and without Night Eating

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### Abstract

**Objective**—To explore appetite-related hormones following stress in overweight individuals, and their interaction with Night Eating (NE) status.

**Method**—We measured plasma cortisol and ghrelin concentrations, and recorded ratings of stress and hunger in response to a physiological laboratory stressor (Cold Pressor Test, CPT) in overweight women with (n=11; NE) and without (n=17; non-NE) night eating.

**Results**—Following the CPT, cortisol ( $p < .001$ ) and ghrelin ( $p < .05$ ) levels increased, as did stress and hunger ratings (all  $p < .001$ ), across all subjects (NE and non-NE). NE exhibited higher baseline cortisol ( $p < .05$ ) levels than non-NE. NE also had greater cortisol area under the curve (AUC) than non-NE ( $p = .019$ ), but not when controlling for baseline cortisol levels. Ghrelin baseline and AUC did not differ between groups. NE showed higher AUC stress ( $p < .05$ ), even when controlling for baseline stress.

**Discussion**—Overweight individuals showed increased cortisol, ghrelin, stress and hunger following a laboratory stressor, and there was some evidence for greater increases in cortisol and subjective stress among NE. The greater AUC cortisol in NE was due to higher baseline levels but the group difference in stress was in direct response to the CPT stressor. Our results support a role for cortisol and stress in Night Eating.

### Keywords

eating disorders; HPA axis; sleep; circadian; appetite hormones; gut peptides

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### Conflict of interest

The authors declare no conflict of interest.

## 1. Introduction

Obesity is usually the result of a prolonged state of energy imbalance from excessive food intake. The eating behaviors that lead to the imbalance, however, may differ widely among individuals. One behavioral phenotype gaining increasing attention is the Night Eating Syndrome (NES), which is characterized by a cluster of behaviors and symptoms, including evening hyperphagia, awakenings from sleep to eat, morning anorexia, and sleep disturbances.<sup>1</sup> Individuals with NES report sleep onset and waking times similar to those without NES, suggesting NES does not represent a disordered biological rhythm that encompasses sleep, but instead a dissociation between the circadian pattern of eating and sleep.

The prevalence of NES has been estimated at 1.5% in the general population,<sup>2</sup> 9–15% among obese treatment-seekers,<sup>3</sup> and 28% among severely obese individuals considering bariatric surgery.<sup>2</sup> Longitudinal evidence<sup>4</sup> and cross-sectional comparisons between obese and lean individuals with NES<sup>5</sup> suggest that night eating is not only more common in obese people, but may also precede the development of obesity, implicating night-time eating as a potential behavioral path to weight gain.<sup>6</sup>

Little is known about the origins of the dysregulated eating patterns in NES. However, life stress has been linked to binge eating,<sup>7, 8</sup> and bulimia nervosa (BN) patients show increases in both desire to binge eat and hunger following interpersonal stress, whereas restrained eaters and normal controls show no increase.<sup>9</sup> Stress may also play a role in NES, which often develops during periods of life stress<sup>10</sup> and remits when stress is alleviated.<sup>11, 12</sup>

The effect of stress on food intake in these eating disorders may be mediated partly by the stress hormone cortisol, which is secreted in response to stress, and may increase intake of high-energy “comfort” foods, possibly contributing to the development of obesity in both humans<sup>13</sup> and animals.<sup>14, 15</sup> For example, Koo-Loeb et al. (2000) found that after an interpersonal speech task, 24-hour urinary cortisol as well as heart rate and blood pressure reactivity were higher in women with BN than women without BN.<sup>16</sup> In our lab, we observed a heightened cortisol response to a physiological stressor in obese women with Binge Eating Disorder (BED)<sup>17</sup> compared with obese controls.

Although primarily known for its role in increasing food intake, the gut peptide hormone ghrelin may also play a role in the stress response and lead to stress-induced eating. Ghrelin is dysregulated in binge eaters<sup>18, 19</sup> and obese individuals,<sup>20</sup> and ghrelin may also be responsive to stress. For example, one study reported increased ghrelin in rats following acute water avoidance stress,<sup>21</sup> while another reported increased ghrelin in humans in response to a social stressor.<sup>22</sup>

Night eaters appear to have elevated levels of cortisol throughout a 24 h period, especially from 8 am through 2 am,<sup>23</sup> although in another study there were no differences.<sup>10</sup> There have not been any studies, however, on the cortisol or ghrelin response to a laboratory stressor in night eaters.

We administered a laboratory stress test to women with and without night eating, and obtained ratings of stress and hunger. To investigate whether the effects of stress could be attributed to cortisol rather than to a more generalized psychological reaction, we used a predominantly physiological stressor, the Cold Pressor Test, which has been shown to raise cortisol more reliably than psychological stressors.<sup>24</sup> Since the stressful events contributing to accumulation of cortisol and consequent nighttime eating are more likely to happen in the day than at night, we conducted the test in the afternoon, when most participants would be at work and/or engaged in their daily routine. To avoid body weight differences confounding cortisol differences between groups,<sup>25,26</sup> we recruited only overweight women. Our first aim was to test whether there would be greater cortisol, ghrelin, stress and hunger following a laboratory stressor in an overweight sample. Our second aim was to test whether overall and baseline cortisol levels would be higher among the NE group. A third aim was to test associations between hormonal responses (cortisol, ghrelin) and subjective ratings (stress, hunger).

## 2. Methods

### 2.1 Participants

Overweight (BMI > 27 kg/m<sup>2</sup>) women were recruited through local advertisements. During an initial telephone interview, candidates were screened to exclude those with significant health problems, including gastrointestinal, heart, kidney, or liver disease, or cancer, hypertension, or diabetes. Additional exclusion criteria included pregnancy or lactation, Raynaud's disease (extreme peripheral sensitivity to cold), unstable body weight ( $\pm$  5%) over the past 3 mo, dieting currently or over the past 3 months, smoking, use of most prescribed medications (especially those affecting body weight, e.g. antidepressants, stimulants), substance abuse or dependence within the past 6 mo, or previous hospitalization for psychiatric illness. A physical examination, including medical history, ECG, and blood tests, was conducted to ensure good health other than obesity, and oral contraceptive use was documented. The study was conducted at St. Luke's-Roosevelt Hospital and the protocol and consent form were approved by the St. Luke's-Roosevelt Hospital IRB.

On the day of screening, participants completed the Night Eating Diagnostic Questionnaire (NEDQ), a 21-item self-report instrument for assessing NES including nocturnal eating.<sup>27</sup> Eleven participants were classified as Night Eaters (NE). Of these 11, 10 reported eating more than 50% of their daily food intake after 7 pm, and 4 reported awakenings from sleep to eat at night, with 3 reporting both. As this was an older version of the NEDQ, the current provisional diagnostic criteria, including consuming 25% of daily food intake after dinner<sup>1</sup> were not applied in the current study. Other related behaviors included difficulty falling or staying asleep (n = 11), morning anorexia (n = 9) or eating breakfast on  $\leq$  3 d/week (n = 7). Another 17 participants reported no late evening or night-time eating behaviors (non-NE).

### 2.2 Procedures

**2.2.1 Anthropometrics and body composition**—Following an overnight 12-h fast, a technician in the Body Composition Lab measured height and weight, to obtain BMI, and waist circumference as an index of central adiposity. Percentage of body fat was assessed by

underwater weighing (Precision Biomedical Systems) to obtain water displacement in order to calculate body volume and density.<sup>28</sup> The technician was blind to participants' night eating status.

**2.2.2 Cold Pressor Test (CPT)**—On a separate day (main test day), participants arrived at the hospital following a 12-hour overnight fast. Self-reported time of last meal was 7:01 pm  $\pm$  1:31 SD, with no significant difference between NE and non-NE groups. Menstrual cycle (number of days from start of menstruation) was recorded since menstrual status might affect cortisol responses to laboratory stress.<sup>29</sup> An IV catheter was inserted into a forearm vein of the non-dominant arm. Following blood draws and consumption of a fixed nutritionally complete liquid meal (600 ml/1254 kcal Boost, Mead Johnson) as part of another protocol, the participants rested for two hours, and then the CPT was conducted at 12:23 pm  $\pm$  1:24 SD. The participant immersed the non-dominant hand up to the wrist in a rectangular shaped container of 0° C ice-water for 2 min. A strainer bag with ice was kept in the water to prevent a rise in temperature. Blood was drawn at -10 and 0 min for baseline measures, at 2 min corresponding to hand withdrawal, and at 5, 15, 30, 45, and 60 min following hand submersion. Participants rated stress and hunger on a visual analogue scale (VAS) from 0 (not at all) to 100 (extremely) immediately before each blood draw. Digital monitors were used to assess diastolic blood pressure, systolic blood pressure and heart rate confirm the occurrence of a stress response.

Blood samples for ghrelin were collected in tubes containing EDTA and aprotinin (Trasyol), kept on ice, and then cold centrifuged for 15 min to obtain plasma, which was stored at -70 C° until assayed. Total cortisol was measured with a radioimmunoassay (RIA) kit from Diagnostic System Labs (intra-assay CV = 2.8; inter-assay = 4.8), and total ghrelin was measured with an RIA kit from Phoenix (intra-assay CV = 2.7, inter-assay CV = 3.2).

**2.2.3 Data analyses**—Sample characteristics were compared between groups (NE vs. non-NE) using univariate ANOVAs and two-tailed t-tests for continuous variables, and Chi-square ( $\chi^2$ ) tests for categorical variables. Missing CPT data were obtained by interpolating between adjacent time-points, or by carrying forward the last value if the final value was missing, and Area Under the Curve (AUC) was calculated for cortisol, ghrelin, and VAS ratings, using the trapezoidal method.

Repeated measures ANOVAs were used to test for change in cortisol, ghrelin, stress and hunger across all subjects. Post-hoc tests, conducted only where there was a significant main effect, examined differences between each of the outcome values and the baseline; averaged (i.e., mean of -10 and 0 min) baseline values were used to give the most representative estimate of the baseline period.

To test for overall group differences between NE and non-NE, we conducted univariate ANOVAs comparing AUC for each outcome; AUC was considered the most appropriate index for group comparisons because cumulative absolute levels of cortisol, ghrelin, stress or hunger in response to day-time stressors are likely to be the most salient predictors of evening eating episodes.<sup>8, 30, 31</sup> To test for baseline differences between NE and non-NE we conducted independent t-tests, also using averaged baseline values. Finally to test whether

group differences were attributable to baseline differences or to overall output following stress, we used univariate ANOVA to test for AUC differences with and without controlling for the initial (i.e. -10 min) baseline value (averaged baseline could not be used because the area between -10 and 0 min contributes to the AUC).

As a secondary test of differences in response magnitude, we also compared peak values for each outcome with and without controlling for averaged baseline. For peak cortisol and ghrelin we took the highest value attained > 2 min, to allow for the time course of these hormone responses. For peak stress and hunger, we took the highest value attained after withdrawal (i.e., at 2 min or thereafter). Finally, Pearson correlations ( $r$ ) were used to test correlations between hormone indices and VAS ratings (averaged baseline, AUC, and peak values) within the whole sample.

All results are presented as mean  $\pm$  SD in Tables and mean  $\pm$  SEM in Figures, with two-tailed  $p < .05$  required for statistical significance and  $p = .06$  for trends. Data were analyzed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL).

### 3. Results

#### 3.1 Participant characteristics

Participants were  $29.7 \text{ y} \pm 7.8 \text{ SD}$  with a BMI of  $35.5 \text{ kg/m}^2 \pm 4.6 \text{ SD}$ , a waist circumference of  $100.9 \text{ cm} \pm 11.2 \text{ SD}$ , and  $40.8 \% \pm 5.2 \text{ SD}$  body fat (from underwater weighing). Twenty-four women (9 NE, 15 non-NE) were obese (BMI  $\geq 30$ ) and 4 (2 NE, 2 non-NE) were overweight (BMI = 27–30). Menstrual phase on the day of testing was available for 19 women (7 NE, 12 Non-NE;  $\chi^2 [1,28] = 0.1$ ,  $p = .51$ ). Of these, 8 were in the follicular phase and 11 in the luteal phase. Only 4 women (2 NE, 2 non-NE) used oral contraceptives, and the proportion of use did not differ by NE status ( $\chi^2 [1,28] = 0.2$ ,  $p = .64$ ). Since none of these variables differed by NE status, they were not included as covariates in further analyses.

#### 3.2 Responses to CPT

##### 3.2.1 Change in cortisol, ghrelin, stress, and hunger following CPT

**Cortisol:** Repeated measures ANOVA revealed that cortisol levels changed significantly following CPT ( $F[7,182] = 10.0$ ,  $p < .001$ ) (Fig 1a). Post-hoc analyses comparing each value with baseline showed that 2 min values were lower than baseline, and 15 and 30 min values higher than baseline (all  $p < .05$ ).

**Ghrelin:** Ghrelin levels increased significantly following CPT ( $F[7,105] = 2.2$ ,  $p = .038$ ) (Fig 1b). Post-hoc analyses showed that values at 5 and 15 min were significantly higher than baseline (all  $p < .05$ ).

**Stress:** Stress ratings also increased following CPT ( $F[7,182] = 35.5$ ,  $p < .001$ ) (Fig 2a), with post-hoc analyses revealing significantly higher levels at 2 and 5 min (all  $p < .05$ ).

**Hunger:** Hunger ratings increased towards the end of the CPT testing period ( $F[7,182] = 12.0, p < .001$ ) (Fig 2b), such that levels at 30, 45 and 60 min were higher than baseline (all  $p < .05$ ).

### 3.2.2 Baseline cortisol, ghrelin, stress, and hunger in NE vs. non-NE—NE

participants had greater baseline cortisol than Non-NE ( $t[26] = 2.7, p = .013$ ). There were no differences in baseline ghrelin, stress, or hunger by NE group.

### 3.2.3 AUC and peak cortisol, ghrelin, stress, and hunger following CPT in NE vs. non-NE

**Cortisol:** NE participants had greater AUC cortisol than non-NE ( $F[1,26] = 5.2, p = .031$ ). This difference became non-significant when controlling for baseline ( $F[1,25] = 0.01, p = .91$ ). There was a trend towards higher peak cortisol in NE ( $F[1,26] = 3.9, p = .058$ ), but not after controlling for baseline ( $F[1,25] = 0.001, p = .97$ ) (Table 1).

**Ghrelin:** AUC ghrelin did not differ between NE and non-NE either before ( $F[1,15] = 0.65, p = .43$ ) or after controlling for baseline ( $F[1,14] = 0.3, p = .57$ ). Peak ghrelin values also did not differ between the groups either before ( $F[1,15] = 1.6, p = .23$ ) or after controlling for baseline ( $F[1,14] = 1.6, p = .23$ ) (Table 1).

**Stress:** NE showed significantly higher AUC stress levels after controlling for baseline ( $F[1,25] = 5.8, p = .023$ ), but not before doing so ( $F[1,26] = 3.7, p = .065$ ). There was a marginal group difference in peak stress between groups both before ( $F[1,26] = 4.2, p = .051$ ) and after controlling for baseline ( $F[1,25] = 4.0, p = .056$ ), such that stress was higher for NE (Table 1).

**Hunger:** NE and Non-NE groups did not differ in AUC hunger ( $F[1,26] < 0.001, p = .999$ ), and this result was unchanged by controlling for baseline ( $F[1,25] = 0.14, p = .71$ ). There were no group differences in peak hunger either before ( $F[1,26] = 0.001, p = .98$ ) or after controlling for baseline ( $F[1,25] = 0.1, p = .76$ ) (Table 1).

**3.2.4 Correlations between hormone indices and VAS ratings—**Pearson's correlations were conducted between baseline, AUC, and peak values for cortisol, ghrelin, stress and hunger. Peak cortisol was positively correlated with AUC hunger ( $r=0.38, p=.049$ ) (Fig 3), but there were no significant relationships between cortisol and stress. There were no significant relationships between ghrelin and either of the VAS indices, or between cortisol and ghrelin indices (all  $p > 0.05$ ).

## 4. Discussion

This is the first study to examine hormonal and psychological responses to a laboratory stressor, in overweight individuals with and without Night Eating. We observed significant increases in cortisol, ghrelin, stress and hunger following the CPT across all subjects. We also observed greater baseline and overall (AUC) cortisol in NE as compared to non-NE, as well as significantly larger overall (AUC) stress after adjusting for baseline values.

Our observations of increased cortisol across groups are consistent with other studies (e.g.<sup>24</sup>), and reflect the efficacy of the Cold Pressor Test. The group difference in overall cortisol output following stress also suggests a role for this stress hormone in night eating, although the higher baseline levels, and the absence of the relationship when controlling for the baseline, suggests that NE is associated with increased baseline cortisol, rather than alterations in the stress response per se. The finding that cortisol in NE shows the normal pattern of a typical stress-induced increase on top of levels which are already elevated is important, though, since overall cortisol levels could have an impact on intake, as well as on metabolic health and weight.<sup>37</sup> One plausible interpretation is that cortisol, which accumulates in response to day-time stress, acts to trigger eating episodes at night, thus helping to maintain the disordered eating and sleep profile observed in NE.

The absence of a group difference in ghrelin may have been due to already low ghrelin levels in these overweight and obese participants, resulting from long-term down-regulation,<sup>20</sup> and making it more difficult to obtain a differential effect. Assaying total rather than active ghrelin may also have obscured the effect, although total and active estimates are highly correlated.<sup>38</sup>

The higher AUC stress ratings (and the trend for higher peak stress ratings) that we observed in the NE group even after controlling for baseline are consistent with greater acute subjective stress rather than higher tonic levels. The results for AUC cortisol and AUC stress vary in so far as the AUC cortisol results support a group difference in basal and overall cortisol, while the AUC stress responses support a difference in acute stress-related increases. Thus, the pattern of higher cortisol in NE may be more pervasive, while differences in psychological responses to stress may be more short-term.

It is unclear why, consistent with other investigators,<sup>39, 40</sup> we did not find correlations between indices of cortisol and subjective stress, or between indices of ghrelin and subjective hunger. This may have been due to a time lag between hormonal and psychological responses,<sup>41</sup> although time-lagged correlations (not reported) did not support this. The time-lagged approach may be more revealing when assessments are made at equal intervals rather than at the uneven time-points in this study.

It is possible that that a direct measure of food intake would have been more sensitive to variations in stress and hormone levels. For example, one study found that women who showed a large cortisol response to cognitivestress consumed significantly more food than those with a smaller response.<sup>42</sup> However, consistent with evidence for a relationship between cortisol and food intake,<sup>13-15, 43</sup> overall cortisol levels were associated with overall levels of hunger across groups suggesting that the AUC measures were sensitive enough to detect this relationship.

CPT was used rather than a social stressor<sup>24</sup> because CPT more consistently induces a rise in cortisol. However, since night eating by definition occurs in the evening, administering the CPT in the evening might have enhanced the observed differences between groups. To thoroughly test the hypothesis that cumulative cortisol and stress leads to increased hunger and night eating, it would also be helpful to obtain free-living measurements for a longer

time period, including evening and night. Other limitations of this study include a relatively small sample size, and the absence of a no-stress control condition. However, the significant sympathetic response to the CPT that we observed here (details not reported) and in other studies<sup>17</sup> indicates that the stress manipulation was effective.

## 5. Conclusion

In summary, this study provided evidence for greater baseline cortisol levels, and greater cortisol AUC following a physiological stressor among overweight women, and particularly among those with night eating. The AUC cortisol difference was apparently driven by higher baseline levels, but the subjective stress difference in response to a laboratory stressor was indicative of greater acute increases among night eaters. These findings are broadly supportive of the hypothesis that elevated baseline and stress-related cortisol could act as maintaining factors for night eating, an increasingly common route to obesity.

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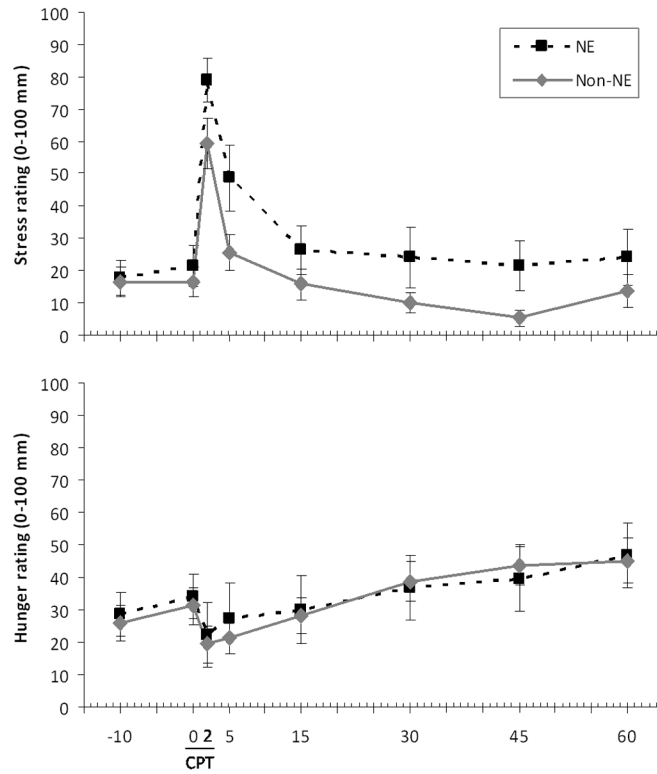
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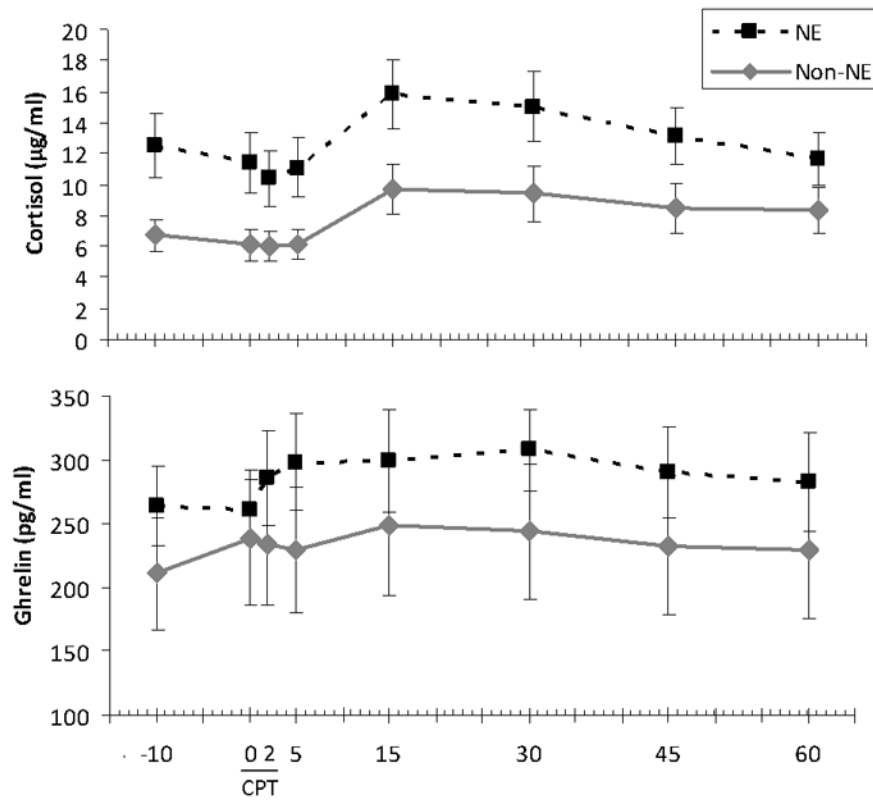


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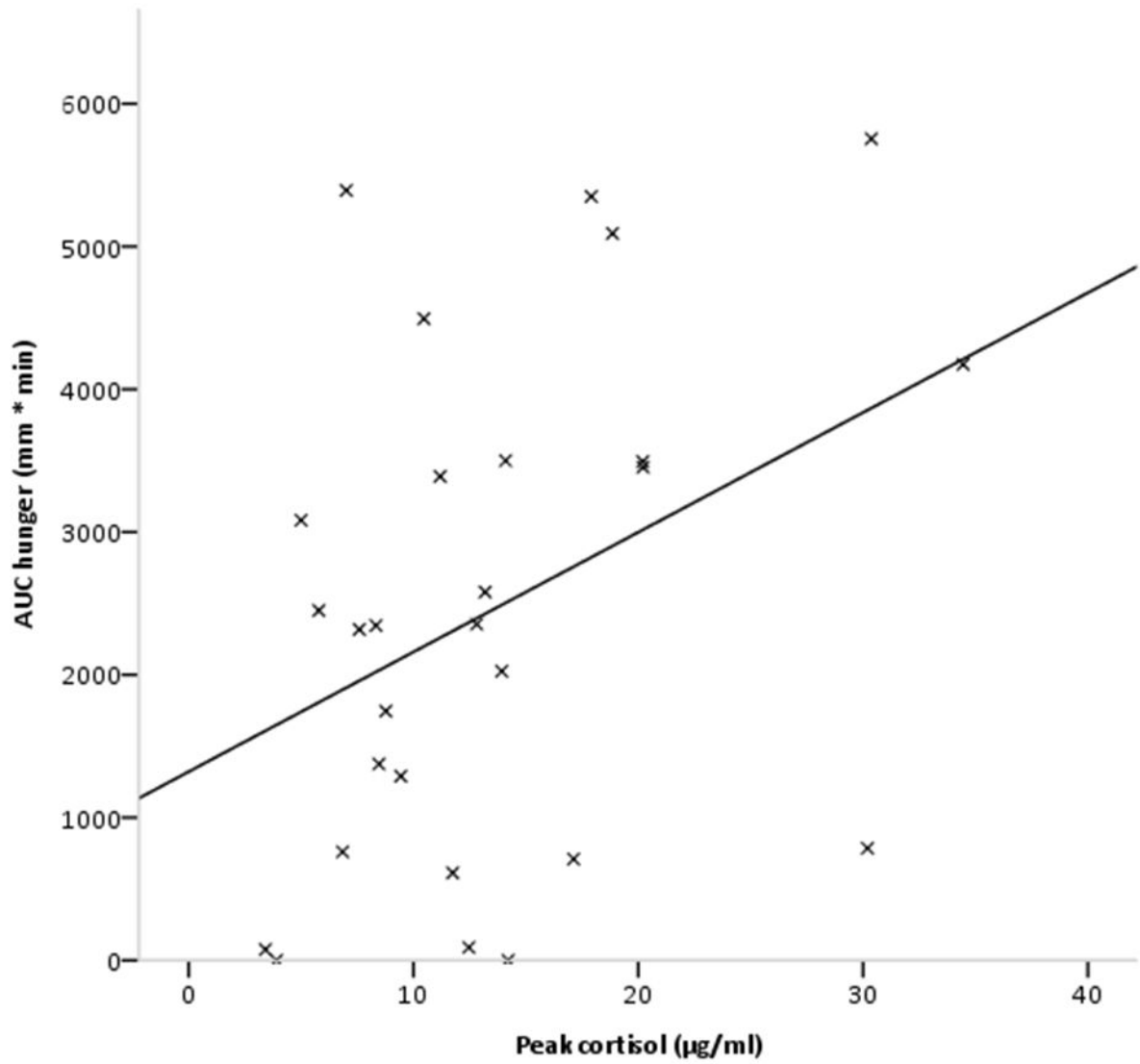
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**Figure 1.** Hormones in relation to Cold Pressor Test in Night Eaters (NE) and non-Night Eaters (non-NE). (a) Cortisol ( $\mu\text{g/ml}$ ), mean  $\pm$  SEM, in response to CPT. (b) Ghrelin ( $\text{pg/ml}$ ), mean  $\pm$  SEM, in response to CPT.



**Figure 2.** Visual Analog Scale (VAS) ratings in relation to Cold Pressor Test in Night Eaters (NE) and non-Night Eaters (non-NE). (a) VAS stress (0 – 100 mm), mean  $\pm$  SEM, in response to CPT by NE status. (b) VAS hunger (0 – 100 mm), mean  $\pm$  SEM, in response to CPT by NE status.



**Figure 3.** Scatterplot showing relationship between peak cortisol and AUC hunger, with regression line ( $r=0.38$ ,  $p<.05$ ).

**Table 1**

AUC and peak (mean  $\pm$  SD) values for hormones and VAS ratings in relation to Cold Pressor Test (CPT) in Night Eaters (NE) and non-Night Eaters (non-NE)

	NE	Non-NE
<b>Cortisol</b>		
Baseline <sup>a</sup> ( $\mu\text{g/ml}$ )	12.0 $\pm$ 6.4	6.4 $\pm$ 4.2*
AUC <sup>b</sup> ( $\mu\text{g/ml} \cdot \text{min}$ )	937.4 $\pm$ 431.1	575.7 $\pm$ 393.9*
Peak <sup>c</sup> ( $\mu\text{g/ml}$ )	17.0 $\pm$ 7.9	11.2 $\pm$ 7.4 <sup>†</sup>
<b>Ghrelin</b>		
Baseline ( $\mu\text{g/ml}$ )	262 $\pm$ 73	215 $\pm$ 139
AUC (pg/ml $\cdot$ min)	20376 $\pm$ 5790	16545 $\pm$ 11532
Peak (pg/ml)	355 $\pm$ 111	263 $\pm$ 169
<b>Stress</b>		
Baseline	19.5 $\pm$ 19.3	16.5 $\pm$ 16.9
AUC (mm $\cdot$ min)	1923 $\pm$ 1682	1019 $\pm$ 786
Peak	80.0 $\pm$ 21.7	59.1 $\pm$ 29.0 <sup>†</sup>
<b>Hunger</b>		
Baseline	31.4 $\pm$ 22.6	28.5 $\pm$ 23.0
AUC (mm $\cdot$ min)	2454 $\pm$ 2126	2453 $\pm$ 1579
Peak	47.7 $\pm$ 33.0	48.1 $\pm$ 28.5

<sup>a</sup>Baseline = averaged (mean of -10 and 0 min) value;

<sup>b</sup>AUC = Area Under the Curve;

<sup>c</sup>Peak = highest value post 0 min;

\* Significant ( $p < .05$ ) difference between groups in unadjusted analyses;

<sup>†</sup>Marginal ( $p < .06$ ) difference between groups in unadjusted analyses.