The acute ghrelin response to a psychological stress challenge does not predict the post-stress urge to eat

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Summary
Ghrelin is a growth hormone and cortisol secretagogue that plays an important role in appetite and weight regulation. It is not known whether ghrelin is involved in the eating response to stress in humans. In the present study we examined the effects of psychologically induced stress on plasma ghrelin levels in patients with binge-eating disorder (BED) (n = 8) and in healthy subjects of normal (n = 8) or increased (n = 8) body mass index (BMI). Volunteers were subjected to the standardized trier social stress test (TSST). Heart rate, blood pressure, serum cortisol, serum prolactin, and plasma ghrelin levels were measured throughout the test. In addition, subjects were requested to rate their feelings of anxiety, tension, urge to eat uncontrollably and desire to eat sweets by means of a visual analog scale both before and after the TSST. There was a significant rise in the systolic blood pressure (p = 0.003) in the study population, reflecting induction of physiological changes by the psychological challenge. Basal ghrelin levels were higher in healthy normal weight (385.4 ± 79 pg/ml) than in obese (170.4 ± 15.7 pg/ml) subjects (p < 0.033). Basal ghrelin levels in patients with BED (240 ± 40.8 pg/ml) were at an intermediate level between thin and healthy obese subjects, but this difference did not attain statistical significance. There were no differences in ghrelin levels throughout the test among the groups after correction for BMI, age and gender. A significant difference in the trend time of ghrelin was revealed when the three groups were analyzed according to their cortisol response to stress. Ghrelin levels increased in cortisol responders whereas no change or a decrease in ghrelin levels occurred in cortisol non-responders (p = 0.038). Furthermore, a positive correlation was found between the change in ghrelin and the change in cortisol during TSST (r = 0.444, p = 0.029) but not between the change in ghrelin and the change in systolic blood pressure. The combined score of stress and anxiety was higher in subjects in the higher quartile of ghrelin response in comparison to the lower
1. Introduction

The obesity pandemic, with its related co-morbidities is a major public health problem in western society (Flegal et al., 2002). The wide availability of high-calorie foods and a sedentary lifestyle has been implicated as contributory factors to the ever increasing rate of obesity. In parallel, there has been immense progress in our understanding of central and peripheral factors that control appetite, satiety, food intake and energy expenditure (Druce and Bloom, 2003). New feeding related peptides such as leptin (Zhang et al., 1994), ghrelin (Nakazato et al., 2001) and others have been isolated. It is becoming increasingly evident that central components of the network controlling food intake are part of the brain reward system and are also involved in the regulation of the stress response (Ueta et al., 2003). For example, corticotrophin releasing hormone (CRH), a central component of the hypothalamo-pituitary-adrenal (HPA) axis activation in response to stress, is a feeding inhibitor (Ueta et al., 2003). On the other hand, endocannabinoid and opioids, both orexigenic substances, are components of the brain reward system (Saper et al., 2002).

Ghrelin is an orexigenic peptide that was originally isolated from the stomach and found to be the endogenous ligand for the growth hormone secretagogue receptor (GHS-R) (Nakazato et al., 2001). Counter-intuitively, though, a negative correlation was found to exist between ghrelin levels and body weight, such that obese subjects have lower plasma ghrelin concentrations than normal and low weight individuals (Tschop et al., 2001). Ghrelin actions on feeding induction involve the activation of neuropeptide Y (NPY) and agouti-related protein (AGRP) neurons in the arcuate nucleus of the hypothalamus (Hewson and Dickson, 2000). Other than the hypothalamus, GHS-Rs have been detected in areas that are not classically involved in the control of feeding, such as the hippocampus and substantia nigra (Guan et al., 1997). Furthermore, projections of ghrelin expressing neurons have been detected in areas outside the hypothalamus, including the amygdala and septum (Cowley et al., 2003). In line with these complex interactions, ghrelin can induce feeding in rats, when directly injected to the ventral tegmental area (VTA), a central part of the mesolimbic reward pathway (Naleid et al., 2005). Recent reports indicate that both ghrelin and leptin are involved in the modulation of feeding behavior through direct effects on midbrain dopaminergic neurons. Ghrelin directly activates dopaminergic neurons in the VTA and increases synapse formation and dopamine turnover in the nucleus accumbens (Abizaid et al., 2006). Indeed, these effects of ghrelin are mirror effects of leptin injected to the same area, which induces inhibition of dopamine neuron firing and reduces food intake (Hommel et al., 2004). Activation of the mesolimbic dopaminergic system is involved in motivated behavior conducted to obtain rewards like food or addictive drugs. The rewarding nature of palatable food may be a central mechanism that ensures the drive for feeding, and thus, survival and maintenance of the species (Saper et al., 2002).

The interconnection between the hypothalamus and areas of the limbic system suggests the existence of a neural circuit that facilitates the cross-talk between emotional states and feeding behavior (Carlino et al., 2004). Direct injection of ghrelin in the hippocampus not only elicited increased food intake, but also induced anxiety like behavior in rats (Carlino et al., 2004). In contrast, systemic leptin treatment reversed the unhedonic behavior of chronically stressed rats, such that when injected directly into the hippocampus it produced an antidepressant effect (Lu et al., 2006).

Stress and negative emotions have been associated with both increased and decreased food intake in men (Geliebter and Aversa, 2003). The mechanism underlying this opposed behavioral responses to similar stressors has not been elucidated. In the general population, high stress levels appear to lead to decreased eating (Stone and Brownell, 1994). Underweight individuals have been reported to eat less than normal and overweight subjects during negative emotional states (Geliebter and Aversa, 2003). In the same line, food craving leading to binge eating in patients with bulimia nervosa were found to be associated with high tension and low mood, but with low hunger levels, thus demonstrating a disconnection between emotional and calorific triggers of feeding in this population (Waters et al., 2001).

It is not known whether ghrelin is involved in the eating response to stress in humans. To further explore this area we set out to examine the effects of a standardized psychological challenge on ghrelin levels and on the self-reported urge to eat in subjects of increased and normal weight, as well as in overweight individuals suffering from binge-eating disorder (BED).
2. Subjects and methods

2.1. Subjects

Twenty four subjects were recruited from the Obesity Clinic and among personnel at the Tel Aviv-Sourasky Medical Center. Eight subjects were obese (body mass index—BMI > 30 kg/m², “obese” group), eight had normal weight (BMI > 18 kg/m² and < 25 kg/m², “NW” group) and eight were obese and suffered from BED (BMI > 30 kg/m², and diagnosis of BED, “BED” group). BED was diagnosed according to criteria from DSM IV-R. Subjects diagnosed with anorexia or bulimia nervosa, and with any psychiatric co-morbidities were excluded from this study. Subjects taking medication affecting the central nervous system such as selective serotonin reuptake inhibitors, tricyclic antidepressants, antiepileptic or antipsychotic drugs were not included in the study. Subjects receiving medication known to interfere with cortisol measurements such as contraceptive pills, or estrogen replacement therapy were also excluded from the study. Ten patients with hypertension, four with hyperlipidemia, and three with type II diabetes mellitus, three with hypothyroidism and one with osteoporosis were well controlled with appropriate medical treatment. The study was approved by the Institutional Review Board and all subjects signed an informed consent form.

2.2. Trier social stress test (TSST)

The TSST is a standardized procedure developed for the induction of moderate psychological stress under laboratory conditions (Kirschbaum et al., 1993). The stressing task consisted of delivering a speech for a job application on the basis of personal characteristics. Psychological stress was added by mock filming the interview for subsequent behavioral analysis. The interview was followed by a mental calculation test.

2.3. Study protocol

Subjects were instructed to eat a light breakfast around 07 00 h. Tests began at 09 00 h and were performed at the Institute of Endocrinology and Metabolism of the Tel Aviv-Sourasky Medical Center. Upon arrival, an intravenous catheter was inserted in the subject’s forearm and after a 10 min rest, pulse and blood pressure were measured, and a blood sample was drawn for hormonal determinations (time −30). At this point, the volunteers answered a short questionnaire regarding their subjective feelings of stress and urge to eat, using visual analog scales (VAS) to rate their answers. The same questionnaire was completed again following the stress test. An additional blood sample was drawn immediately before the beginning of the test (time 0). The stress period lasted about 15 min after which a third blood sample was drawn. Subjects were taken then to a rest area, where blood was sampled again 20, 40 and 60 min after the completion of the stress test. Blood pressure and pulse rate were measured at the same time points.

2.4. Visual analog scales

Subjects were requested to grade their feelings before and after the stress test using scales ranging from 0 (not at all) to 100 (very much), answering specifically to the following questions:

(1) To what extent do you feel now an urge for uncontrolled eating?
(2) To what extent do you feel now craving for sweet foods?
(3) To what extent do you feel stressed now?
(4) To what extent do you feel anxiety now?

2.5. Laboratory measurements

Blood samples were collected in pre-chilled tubes containing EDTA (1 mg/ml) and aprotinin (500 U/ml; Phoenix Pharmaceuticals, Belmont, CA, USA) and immediately centrifuged. Plasma was stored at −80 °C until assayed. Human total plasma ghrelin was measured with a commercial radioimmunoassay (Phoenix Pharmaceuticals) with an inter-assay coefficient of variation (CV) of <12% and an intra-assay CV of <7%. Blood was also collected for serum measurements of cortisol (Electrochemiluminescence Immuno Assay “Elecsys 2010”, Roche; within run precision CV 1.4% and between run CV 2.1%) and prolactin (Chemiluminescence Immuno Assay, Immulite 2000; within run precision CV 2.3% and between run CV 5.9%).

2.6. Statistical analysis

Results are given as mean ± SEM. Basal hormone levels (baseline) were calculated as the mean of measurements obtained at time points −30 and 0. The parameter “change” in all parameters following stress was computed as the difference between the mean values obtained at four time points after TSST and the pre-stressor baseline [change = (T1+T2+T3+T4)/4 − (T−30+T0)/2]. Area under the curve was calculated following the trapezoid formula. Comparisons of baseline continuous parameters (age, BMI, heart rate, blood pressure and hormone pre-stressor concentrations) between groups (obese, thin and BED) were performed by a one-way analysis of variance with multiple comparisons using the Ryan–Einot–Gabriel–Welsch Multiple Range Test. Groups were compared with respect to gender by Fisher’s exact test. The effect of TSST on blood pressure, heart rate, and cortisol, prolactin and ghrelin levels was examined by way of ANOVA with repeated measures. Comparisons of post-stress curves of ghrelin, prolactin, cortisol, heart rate and blood pressure between groups was performed by two-way ANCOVA with repeated measures. Gender and group (NW, obese, BED) were entered as grouping variables whereas age and BMI were included as covariates. Further comparison between groups included the classification of patients into cortisol responders and non-responders. Whenever a positive change (as defined above) in cortisol levels was recorded, patients were considered as cortisol responders. This parameter was included in the ANCOVA with repeated measures as specified.
above. Associations between continuous parameters were evaluated by calculating Pearson correlation coefficients adjusted for BMI, age and gender. Pre- and post-stress changes in parameters measured by VAS were compared by Wilcoxon signed rank test, and these differences were compared between groups by way of Wilcoxon rank-sum test. Multivariate logistic regression model was applied to the data to study the possible explanatory variables for change in ghrelin. This variable was coded using two levels of cut off: $1 = $ ghrelin change $>$ 5, and $0 = $ ghrelin change $<5$; or $1 = $ ghrelin change $>$ 21 and $0 = $ ghrelin change $<21$. Parameters considered for the model were gender, age, BMI, baseline ghrelin and change in cortisol. The same model, with the addition of the parameter change in ghrelin was applied to the data to study the possible explanatory variables for change in urge to eat (UE). This variable was coded into $1 =$ increase and $0 =$ decrease or no change in UE. Additional analysis involved the classification of subjects according to quartiles of ghrelin change. The upper quartile ($n = 6$) composed of subjects with ghrelin change $>30$ and the lower quartile ($n = 6$) composed of subjects with ghrelin change $<-5$. Comparisons between the two extreme quartiles with regard to parameters measured on VAS were performed using the Mann–Whitney non-parametric test. Comparisons between the two extreme quartiles with regard to cortisol response were performed by two-way ANCOVA with repeated measures as specified above. All statistical analyses were carried out using the SAS for Windows version 9.1.

3. Results

3.1. Baseline characteristics of the study population

3.1.1. Demographics

Twenty-four subjects (mean age $44 \pm 3.1$, range 23–70 years) participated in the study.

There were 16 women and eight men, with similar male to female ratios in the three groups. Mean age was significantly lower in the NW group ($32.6 \pm 3.5$ years) in comparison with the obese ($50.2 \pm 5.7$ years) and BED ($49.7 \pm 4.8$ years) groups [$F(2,21) = 4.39$, $p = 0.026$].

3.1.2. Physiological and hormonal parameters

Heart rate, systolic and diastolic blood pressure, serum cortisol and prolactin levels at baseline were similar in the three groups. As expected, basal plasma ghrelin levels were higher in the NW group ($385.4 \pm 79$ pg/ml) than in the obese ($170.4 \pm 15.7$ pg/ml) group [$F(2,21) = 4.04$, $p = 0.033$]. Basal ghrelin levels in patients with BED ($240.5 \pm 40.8$ pg/ml) were at an intermediate level between thin and healthy obese subjects, but this difference did not attain statistical significance. Characteristics of the study population are detailed in Table 1.

3.1.3. Psychological parameters

No significant differences were found in mean baseline VAS scores for stress, anxiety, and craving for sweets among the three groups. In contrast, VAS score for the urge for uncontrolled eating (UE score) was higher in the BED group than in the NW and obese groups ($p = 0.03$) at baseline (Table 2).

3.2. Validation of TSST as an acute stressor in the study population

3.2.1. Psychological parameters

There was a significant increase in the subjective feeling of stress after TSST according to the VAS in the population study (stress score $21.8 \pm 5.4$ before and $40.6 \pm 5.3$ after TSST; $n = 24$, $p < 0.0001$). Anxiety scores were low in general, nevertheless a significant rise was observed following the psychological stress ($9.8 \pm 3.2$ at baseline, $19 \pm 5$ after stress; $n = 24$, $p = 0.002$). TSST induced changes in stress and anxiety scores were also analyzed for each individual group ($n = 8$; Table 2). Stress scores significantly increased in the NW and BED groups, but the increase that occurred in the obese group was not significant. Increase in anxiety scores did not attain statistical significance when analyzed separately for each group, most probably reflecting the smaller sample size.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of the study population.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thin ($n = 8$)</td>
</tr>
<tr>
<td></td>
<td>mean $\pm$ SEM</td>
</tr>
<tr>
<td>Age (years)</td>
<td>$32.6 \pm 3.5$</td>
</tr>
<tr>
<td>Male female ratio (M/F)</td>
<td>3:5</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>$21.2 \pm 0.7$</td>
</tr>
<tr>
<td>Heart rate</td>
<td>$70 \pm 3.7$</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>$112 \pm 5.2$</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>$69 \pm 3$</td>
</tr>
<tr>
<td>Basal plasma ghrelin level (pg/ml)</td>
<td>$385.4 \pm 79$</td>
</tr>
<tr>
<td>Basal serum cortisol level (µg/dl)</td>
<td>$12.4 \pm 2.3$</td>
</tr>
<tr>
<td>Basal serum prolactin level (µg/ml)</td>
<td>$6.5 \pm 0.9$</td>
</tr>
</tbody>
</table>

NS, not significant; BED, binge eating disorder.

$^a$Thin vs. obese and BED.

$^b$Thin vs. obese.

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Heart rate during the test; subjects there was a significant decrease in cortisol levels are at odds with the robust increase in cortisol induced by a significant increase in cortisol levels, which significantly affected the physiological diurnal variation (‘‘cortisol responders’’, without the expected decrease related to the physiological response to the psychological stress in the study population. Heart rate remained stable but slowed down towards the completion of the stress test. 13 of 24 subjects responded to the psychological stress with either a significant increase in cortisol response. Thirteen of 24 subjects responded to the psychological stress test. Taken that other parameters examined in this study could depend on or be affected by the TSST effect on cortisol, we also performed subsequent analyses according to the expected diurnal variation (‘‘cortisol non-responders’’).

### 3.2.2. Physiological parameters

There was a significant increase in systolic blood pressure \([n = 24, F(4,20) = 5.8, p = 0.003 , \text{ Fig. 1}],\) reflecting the physiological response to the psychological stress in the study population. Heart rate remained stable but slowed down towards the completion of the stress test \([n = 24, F(4,20) = 3.9, p = 0.015 , \text{ Fig. 1}].\)

TSST did not induce an increase in prolactin levels, which gradually decreased during the test \([n = 24, F(4,20) = 5.25, p = 0.0047 , \text{ Fig. 2}].\) TSST induced only a modest and non-significant increase in cortisol levels, which significantly decreased according to the expected diurnal variation \([n = 24, F(4,20) = 10.14, p = 0.0001 , \text{ Fig. 2}].\) These results are at odds with the robust increase in cortisol induced by TSST described in previous reports (Kudielka et al., 2004). Taken that other parameters examined in this study could depend on or be affected by the TSST effect on cortisol, we also performed subsequent analyses according to the cortisol response. Thirteen of 24 subjects responded to the psychological stress with either a significant increase in cortisol levels, or with sustained stable cortisol levels, without the expected decrease related to the physiological diurnal variation (“cortisol responders”, \(n = 13, F(4,9) = 6.07, p = 0.0119 , \text{ Fig. 3}].\) In the 11 remaining subjects there was a significant decrease in cortisol levels during the test \([n = 11, F(4,7) = 11.41, p = 0.0035 , \text{ Fig. 3}]\) according to the expected diurnal variation (“cortisol-non-responders”).

### 3.3. Effect of TSST on ghrelin levels

There was no significant change in ghrelin levels over time throughout the stress test in the population study as a whole. Ghrelin levels were higher throughout the test in the NW compared to obese and BED groups \([F(1,22) = 6.09, p = 0.022 , \text{ Fig. 4}].\) However, there were no differences among the groups after correction for BMI, age and gender, nor there was any change in ghrelin over time in each individual group.

#### Table 2  Visual analog scale scoring for the specific parameters before (0) and after (1) the psychological stress test in the study population.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All ((n = 24))</th>
<th>Normal weight</th>
<th>Obese</th>
<th>Obese and BED</th>
<th>(p) value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress 0</td>
<td>21.8 ± 5.4</td>
<td>17.5 ± 5.2</td>
<td>23.1 ± 10.6</td>
<td>25 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>Stress 1</td>
<td>40.6 ± 5.3</td>
<td>40 ± 7.7</td>
<td>35.6 ± 10</td>
<td>46 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>(p) value&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>0.01</td>
<td>NS</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Anxiety 0</td>
<td>9.8 ± 3.2</td>
<td>5 ± 2.6</td>
<td>9.3 ± 9</td>
<td>15 ± 7</td>
<td>NS</td>
</tr>
<tr>
<td>Anxiety 1</td>
<td>19 ± 5.5</td>
<td>18 ± 7.8</td>
<td>14.3 ± 7.1</td>
<td>23.7 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>(p) value&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.002</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Sweets 0</td>
<td>8.1 ± 2.8</td>
<td>6.2 ± 3.7</td>
<td>1.8 ± 1.3</td>
<td>16.2 ± 6.8</td>
<td>NS</td>
</tr>
<tr>
<td>Sweets 1</td>
<td>12.9 ± 4.2</td>
<td>6.2 ± 2.6</td>
<td>5 ± 2.6</td>
<td>27.5 ± 10.7</td>
<td>NS</td>
</tr>
<tr>
<td>(p) value&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Urge to eat 0</td>
<td>8.5 ± 3.5</td>
<td>2.5 ± 2.5</td>
<td>1.9 ± 1.3</td>
<td>21.2 ± 8.9</td>
<td>0.03</td>
</tr>
<tr>
<td>Urge to eat 1</td>
<td>16.2 ± 5.2</td>
<td>8.7 ± 6</td>
<td>7.5 ± 1.8</td>
<td>35 ± 12</td>
<td>0.02</td>
</tr>
<tr>
<td>(p) value&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.012</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>For comparisons among the three groups (NW, obese and obese with BED).

<sup>b</sup>For comparisons between values pre- and post-TSST.

#### Figure 1  Systolic blood pressure and heart rate in the study population before, during and following the psychological stress test. \(p = 0.003\) for changes in systolic blood pressure and \(p = 0.015\) for changes in heart rate by repeated measures ANOVA. *\(p<0.001\) vs. baseline. Arrow denotes the time period of the actual stress.

Analysis of variance was repeated taking into consideration the cortisol response in each individual group, with adjustments for BMI, age and gender. There was a significant difference in the change of ghrelin over time in that ghrelin levels increased in the group of cortisol responders but not in the group of cortisol non-responders \([F(4,12) = 3.59, p = 0.038 , \text{ Fig. 5}].\) The pattern of ghrelin response was not significantly different among the three original groups \([F(8,24) = 2.21, p = 0.068 , \text{ Fig. 5}].\)

### 3.4. Determinants of ghrelin response

To further explore possible determinants of ghrelin response to TSST, subjects were divided according to quartiles of anxiety, urge to eat, psychoneuroendocrinology (2007), doi:10.1016/j.psyneuen.2007.04.010
change in ghrelin (computed as the difference between the mean of four post-stress ghrelin measurements and the pre-stressor baseline, Fig. 6A). The upper and lower quartiles were compared regarding the change in cortisol levels, with adjustments for gender, age and BMI. As shown, in the lower quartile of ghrelin response, cortisol levels decreased steadily following TSST, thus likely representing a normal, uninterrupted diurnal decline. In contrast, in subjects at the highest quartile of ghrelin increment following TSST, serum cortisol initially increased, declining thereafter to levels lower than baseline cortisol. Time trend was significantly different in the two extreme quartiles (F(4, 28) = 2.98, p = 0.04, Fig. 6B), supporting the existence of an interaction between cortisol and ghrelin responses. Furthermore, a positive correlation was found between the change in ghrelin and the change in cortisol during TSST (r = 0.444, p = 0.029; Fig. 7) but no correlation was found between the change in ghrelin and the change in systolic blood pressure. Adjustment for age, BMI and gender weakened the correlation between change in ghrelin and change in cortisol (r = 0.403, p = 0.07). Nevertheless, we did not find alternative, independent explanatory variables for the change in ghrelin in a multivariate logistic regression model.

3.5. Effect of TSST on eating parameters

TSST induced a significant increase in UE score in the study population (8.5 ± 3.5 before and 16.2 ± 5.2 after TSST, respectively, n = 24, p = 0.012). It should be noted, though, that most subjects (n = 13) had a UE score of zero both before and after TSST, and only nine of 24 subjects reported an increase in their urge to eat after the psychological stress (UE score 13.8 ± 7.4 before and 36 ± 10 after the test, n = 9, p = 0.004). Although the UE score was significantly higher in the BED group in comparison with the NW and obese groups after TSST (p = 0.02, Table 2), the nine subjects reporting an actual increase from baseline in their UE score were evenly distributed among the three study groups (three NW, two obese and four BED). The area under the curve of ghrelin was marginally higher in these nine patients (ghrelin AUC 1385 ± 273 vs. 875 ± 112 pg h/ml; p = 0.053). No independent explanatory variable was found in a multivariate logistic regression model for increase in UE score. Additionally, TSST did not induce a significant change in the desire to eat sweets score.

3.6. Interrelation between ghrelin response to TSST and reported subjective parameters

The combined score of stress and anxiety was higher in subjects at the higher quartile of ghrelin response in comparison to the lower quartile both before and following TSST. p = 0.0001 for decrease in cortisol and p = 0.0047 for decrease in prolactin by repeated measures ANOVA. Arrow denotes the time period of the actual stress.
On the other hand, eating related scores did not differ according to quartiles of ghrelin change.

4. Discussion

In this study we have shown for the first time that a psychological stress may induce an increase in plasma ghrelin levels in humans. Individuals in the higher quartile of ghrelin response also reported higher subjective scores for psychological stress. In contrast, the post-stress increase in urge to eat that occurred in some individuals was unrelated to acute changes in plasma ghrelin levels. It is interesting to note that this disconnection between the acute ghrelin response to stress and the post-stress urge to eat was also true for patients with BED, in whom binge episodes are often attributed to stress. The stress induced increase in plasma ghrelin was associated with the serum cortisol response. Furthermore, there was a positive correlation between the degree of increase in cortisol and ghrelin levels over baseline, secondary to the stress test. The absence of a correlation between the degree of increase in systolic blood pressure and rise in plasma ghrelin suggests that the HPA axis but not the sympathetic response may mediate the ghrelin response to the psychological stress. At present we cannot explain either the basis of ghrelin increase in just a fraction of subjects, or the significance of its correlation with the cortisol response in humans. Further studies are needed to clarify these issues.

Our results concur with similar investigations performed on rodents. It has been shown that the tail pinch stress, a well established inductor of corticosterone stress response in mice, induced an increase in ghrelin gene expression in the stomach oxyntic mucosa (Asakawa et al., 2001). Subsequently, Kristensson et al. (2006) reported that water immersion elicited a significantly larger increase in plasma ghrelin levels in the high-anxiety Wistar Kyoto strain than in the low anxiety Sprague–Dawley rats. In both strains there was a similar increase in stress-induced ACTH plasma levels. Interactions between ghrelin, HPA axis and the anxiogenic response to a stress stimulus were nicely demonstrated by Asakawa et al. (2001): intraventricular and intraperitoneal injection of ghrelin increased CRH mRNA expression in the hypothalamus, and the concomitant administration of a CRH receptor antagonist inhibited the ghrelin induced anxiogenic behavior. Neither study reported the rodents’ feeding response to the psychological stress.

The interaction between ghrelin and cortisol has been previously explored in humans. Ghrelin administration was shown to induce an increase in plasma ACTH and serum cortisol levels (Arvat et al., 2001; Tassone et al., 2003). Ghrelin's ability to stimulate ACTH is preserved and even enhanced in hypercortisolemic patients (Leal-Cerro et al., 2002). On the other hand, basal ghrelin levels were reportedly decreased in patients with Cushing’s disease and increased after successful treatment, probably reflecting...
the long term influence of cortisol excess on body weight (Otto et al., 2004; Libe et al., 2005). In healthy people, no association has been found between ghrelin and cortisol levels (Purnell et al., 2003), but an inverse correlation between these hormones emerges following prolonged fasting (Espelund et al., 2005). Patients with anorexia nervosa have higher ghrelin levels relative to healthy adolescents. Further, frequent sampling for ghrelin and cortisol revealed a positive association between several secretory features of ghrelin and cortisol such as AUC and the secretory burst frequency in both groups (Misra et al., 2005). In fact, valley ghrelin independently predicted cortisol burst frequency and was responsible for 52% of its variability (Misra et al., 2005). Although this report is in line with our findings, it cannot be presupposed that the positive correlation between ghrelin and cortisol will be retained during the response to the acute psychological stress addressed in our study.

Possible effects of eating disorders on ghrelin levels have been extensively studied. Fasting plasma levels are extremely elevated in patients with anorexia nervosa, reflecting their very low BMI, but, intriguingly, ghrelin levels in these patients were also higher than those found in BMI-matched controls without eating disorders (Otto et al., 2004; Tolle et al., 2003). Ghrelin levels were also found to be higher in bulimia nervosa patients in comparison with weight matched controls (Tanaka et al., 2002). Tanaka et al. (2003) found a positive correlation between the frequencies of binge/purge cycles and ghrelin values and thus suggested that binge/purge behavior may influence circulating plasma ghrelin. In contrast, ghrelin levels were reported to be lower in obese women with BED than in women with matched BMIs free of eating disorders (Geliebter et al., 2005). Monteleone et al. (2005) reported decreased ghrelin levels in both obese and non-obese women with BED, whereas Troisi et al. (2005) concluded that ghrelin concentrations reflect nutritional status irrespective of the type of eating disorder. Our work is in line with this last observation, as we did not find significant differences in ghrelin levels between obese with or without BED, neither at baseline nor following the psychological stress.

A possible limitation of our study is that the psychological stress did not induce a HPA axis response in 11 subjects. The fact that healthy individuals react differently to stressful stimuli such as psychological stress tests and high intensity exercise has been previously reported (Singh et al., 1999). Subjects had been classified as “high and low responders” according to the extent of their cortisol response to stressors (Kirschbaum et al., 1995), although it seems that instead of a categorical cut off, there is rather a continuum of cortisol responses, ranging from those who do not react to any psychological test to those who react to all (Berger et al., 1987).

Our study may be limited also in that we measured total ghrelin levels only. Most information gathered to date on ghrelin effects and regulation is based on the measurements of total ghrelin (acylated and non-acylated forms) in the plasma, as well as on the study of effects of total ghrelin infusion in animals and humans. The acylated form exerts its metabolic and neuroendocrine actions through activation of the GH secretagogue receptor 1a (GHS-R1a). Although the non-acylated form does not bind to GHS-R1a and has been...
Previously considered inactive, it actually does share some non-endocrine actions (e.g., cardiovascular effects) with acylated ghrelin (Broglio et al., 2004). We cannot speculate on whether a stress challenge could differentially affect acylated and non-acylated ghrelin.

Considering the high complexity of both the feeding and the stress responses, it is reasonable to assume that additional factors not measured in this study could have been induced by acute psychological stress and may have affected the subjective urge to eat in our subjects. Furthermore, variable sensitivity to the stimulatory effect of ghrelin on feeding may also be playing a part. Although ghrelin increases food intake in obese as well as in lean subjects, the former are more sensitive to the appetite stimulating effect of ghrelin in that they respond markedly more to lower doses of infused ghrelin compared to normal weight individuals (Druce et al., 2005).

In summary, we have shown that a psychological stress may induce an increase in plasma ghrelin levels in some human subjects, and that the post-stress induced urge for uncontrolled eating is not acutely modulated by stress related elevations in ghrelin levels. Furthermore, the stress induced increase in plasma ghrelin was associated with an acute response of serum cortisol to stress. Finally, the ghrelin response to the psychological challenge was independent of BMI or the presence of BED.

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Conflict of interest

All authors declare that they have no conflicts of interest that could inappropriately influence or be perceived to influence their work.

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