

## Gender differences in ghrelin response to chronic immobilization stress in rats: possible role of estrogen

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**Abstract.** Ghrelin is a peptidergic hormone known to be one of the main hormones involved in the regulation of energy balance. Here we evaluated ghrelin response to stress in rats after ovariectomy and during estradiol benzoate (EB) therapy and compared results of males and females, to know whether ghrelin is involved in disordered eating behaviors in response to stress, and for understanding differences between males and females in food intake and weight gain especially during stress. 96 adult rats were classified into; male, female, ovariectomized (Ovx), Ovx with EB. Half animals of each group exposed to immobilization stress 20 min/day for 21 days. We found that chronic stress significantly augments serum ghrelin levels in both males and females, which is correlated with an increase in food intake and body weight. Females displayed significant higher ghrelin than males especially in response to stress, ovariectomy suppresses serum ghrelin in both unstressed and stressed females which is rescued by replacement with EB. EB replacement augments ghrelin response to stress in Ovx female, and reduces food intake and body weight. Conclusion: There is a clear sex difference in ghrelin secretion in response to stress caused by EB, since it amplifies ghrelin response to stress in females.

**Key words:** Ghrelin — Estrogen — Stress — Gender differences

### Introduction

Being male or female is one of the most important predictors of an individual's health. Compared with women of similar age, men have a higher risk of atherosclerosis (Kalinand Zumoff 1990) and infectious disease (Klein 2000), while women outnumber men for several autoimmune disorders, fibromyalgia and chronic pain (Whitcare et al. 1999). Recently, sex differences in the physiological response to stress have emerged as a potentially important risk factor for stress related disorders. This raises the possibility that sex differences in prevalence of disease could at least in part be explained by sex differences in the nature of the physiological response to stress (Kajantie and Phillips 2005).

One of these disorders which can be triggered by stress is disordered eating behaviors (DEB) which include: vomiting, laxative use, binge eating and frequent dieting (Ackard et al. 2003; Eaton et al. 2006), as a way to cope or reduce negative

emotions (Stice et al. 1996; Loth et al. 2008). These behaviors are of public health concern due to their association with adverse physical and psychological outcomes, including depressive symptoms (Johnson et al. 2002), and the onset of obesity (Stice et al. 2005; Neumark-Sztainer et al. 2006) and eating disorders (Patton et al. 1999; Santonastaso et al. 1999).

In humans, the literature shows that stress affects eating in a bidirectional way; a subgroup, possibly around 30%, decreases food intake and loses weight during or after stress, while most individuals increase their food intake during stress (Stone and Brownell 1994; Epel et al. 2004). Almost 50% of a US representative sample is concerned with the amount of stress in their life copes by engaging in unhealthy behaviors such as smoking as well as eating for relief (Stambor 2006). Another survey study shows increased food intake during times of stress (Zellner et al. 2006). Animal studies reveal that stress can lead in some cases to increase but mainly to decrease in food intake (Levine and Morley 1981; Morley et al. 1983).

Since adequate regulation of energy and food intake under stress is important for survival, it is not surprising

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that the hypothalamic-pituitary-adrenal (HPA) axis is not only the 'conductor' of an appropriate stress response, but is also tightly intertwined with the endocrine regulation of appetite, and it is at least one of the central players in explaining changes, both undereating and overeating due to stress (Adam and Epel 2007).

The physiological regulation of food intake is a complex homeostatic process that is regulated by many endocrine factors. One of these factors is ghrelin, which is a peptidergic hormone mainly secreted by the stomach and known to be one of the main hormones involved in the regulation of energy balance by increasing food intake and reducing fat utilization (Wren et al. 2001). Moreover, ghrelin regulates glucose metabolism (Patel et al. 2006) and possibly is involved in the regulation of insulin activities in man (Murata et al. 2002). Likewise, ghrelin appears to be related to the regulation of energy expenditure (Maffei et al. 2006).

The regulation of gastric and circulating forms of ghrelin has been initially documented largely in relation with the nutritional status (Kojima and Kangawa 2005). Recently, alterations of circulating ghrelin by environmental and visceral stressors (Stengel et al. 2010) and the potential role of ghrelin in the stress response have received growing attention (Patterson et al. 2010). Spencer et al. (2012) found that ghrelin reduces anxiety after acute stress by stimulating the HPA axis at the level of the anterior pituitary, since it targets the growth hormone secretagogue receptor (GHSR) to stimulate adrenocorticotropic hormone (ACTH) release from the anterior pituitary and coordinates central input to the HPA axis from the medial amygdaloid nucleus (MeA) and Edinger-Westphal nucleus (EWcp). Thus, ghrelin regulates acute stress and offers potential therapeutic efficacy in human mood and stress disorders.

The present study was conducted on a trial: (1) to compare the response of ghrelin to immobilization stress between intact males and females and (2) to evaluate ghrelin's response to immobilization stress in ovariectomized female rats with or without estradiol benzoate hormone therapy. These data suggest that ghrelin is involved in the disordered eating behaviors in response to stress, which also involves sex differences in serum ghrelin concentrations and in regulation of food intake and weight gain during stress.

## Materials and Methods

### *Ethical approval*

The local ethics committee in our university, approved this animal experiment protocol, and it was conducted in compliance with the NIH Guide for Care and Use of Laboratory Animals (National Institutes of Health, 1992).

### *Experimental groups and animals*

Ninety-six adults, 8–10 weeks, Sprague Dawley albino rats (24 male and 72 female) weighing between 200–250 g were used throughout the present study. Rats were housed at room temperature with 12 hour light/dark cycle with a supply of a standard diet of commercial rat chow and water ad libitum. Animals were left to acclimatize to the environment for two weeks prior to inclusion in the experiment. The rats were divided into 4 different groups ( $n = 24$ ):

- Group 1: male rats (M) – rats which underwent sham surgical procedure 4 weeks before inclusion in the experiment with sc injection of vehicle (sesame oil) for 3 weeks starting 1 week after the sham operation
- Group 2: female rats (F) – rats which underwent sham surgical procedure 4 weeks before inclusion in the experiment with sc injection of vehicle (sesame oil) for 3 weeks starting 1 week after the sham operation
- Group 3: female rats with bilateral ovariectomy (Ovx) – bilateral ovariectomy was done 4 weeks before inclusion in the experiment with sc vehicle injection for 3 weeks starting 1 week after the ovariectomy
- Group 4: female rats with bilateral ovariectomy treated with estradiol benzoate (Ovx+E) – bilateral ovariectomy was done 4 weeks before inclusion in the experiment with estradiol benzoate supplementation (25 µg/kg/day; SC) for 3 weeks starting 1 week after the ovariectomy (Yu et al. 2009).

Half the animals of each group were exposed to immobilization stress 20 min/day for 21 days. Control male and female group exposed to stress 1 week after the sham operation, for 21 days before animals decapitation, ovariectomized rats exposed to stress 1 week after the ovariectomy, for 21 days before animals decapitation. The animals received the appropriate treatment at the same time every day.

### *Chemicals and kits used*

Estrogen (for the treated ovariectomized group): in the form of ampoules, each ampoule contains estradiol benzoate in oily solution. Sesame oil (for the non-treated male and female groups): in the form of oily solution. Ether (anaesthetic agent for ovariectomy): in the form of diethyl ether LR (C<sub>4</sub>H<sub>10</sub>O). Kits for measuring serum ghrelin and serum corticosterone levels.

### *Ovariectomized rat model*

Ovariectomy in rats is a good model of estrogen insufficiency. The rat was anaesthetized by ether inhalation. The anaesthetized rat was placed on the operating board in dorsal recumbency with its tail directed towards the surgeon. The ventral aspect of the lumbar region was shaved, and then cleaned with 75% ethanol, followed by thorough scrubbing with 10% povidone iodine

(Betadine). 1 cm long longitudinal ventral midline incision was made above the symphysis pubis by a scalpel blade; the skin edges were laterally retracted, and the abdominal muscle layer and the peritoneum were incised. Both fallopian tubes were exposed and ligated; the ovary can usually be seen embedded in a pad of fat in the abdomen; then the ovaries were removed by cutting them with scissors, taking care not to rupture the ovarian capsules. The remaining tissues were replaced into the peritoneal cavity. The incision was then closed using a sterile 2/0 suture. The removed tissues were ensured to be the ovaries by histological sections (Flores et al. 2008).

#### Immobilization stress

Rats assigned to the stress groups were immobilized for 20 min/day for 21 days, rats were restrained by fixing all their limbs to a wood board. The rats were immobilized at the same time each day. Rats were tightened until the animals were immobilized but not compressed, pinched, or in pain. This allows for very little movement. Animals were unable to turn or barrel-roll. This restraint procedure is associated with emotional distress. The duration of immobilization stress has been shown to be sufficient to produce marked elevations in corticosteroids, which is consistent with a physiologic stress response (Bielajew et al. 2002). In this study, measurement of serum corticosterone levels confirmed stress exposure.

#### Body weight and food intake

Body weight and food intake were taken daily over the entire length of the study. Food intake was measured by placing constant amount of food in each cage daily and estimating the remaining amount on the next day. Rats were individually weighed, while food intake was recorded for each group.

#### Animal sacrifice and sample collection

On the final day of the experiment rats were sacrificed by decapitation without anesthesia after 10 h fasting. The blood samples were immediately collected in 10 ml eppendorf tubes, allowed to clot, and then delivered into centrifuge tubes to be centrifuged at 3.000 rpm for 20 minutes; serum samples were separated in 2 ml eppendorf tubes to be used immediately as fresh samples (preferred) or to be stored at  $-20^{\circ}\text{C}$  until used. Rats exposed to immobilization stress were sacrificed 5 min following cessation of the stressor.

#### Parameters measured

At the end of the experimental protocol, the following parameters were measured: serum corticosterone by: rat corticosterone ELISA kit from MyBioSource company, serum ghrelin by: ratghrelin ELISA kit from MyBioSource company.

#### Statistical analysis

All values are presented as means  $\pm$  SEM. Statistical significance was tested using two-way ANOVA test. Analyses were performed using statistical software (SPSS version 11.0; Chicago, IL, USA), and independent samples t-test;  $p < 0.05$  being considered statistically significant.

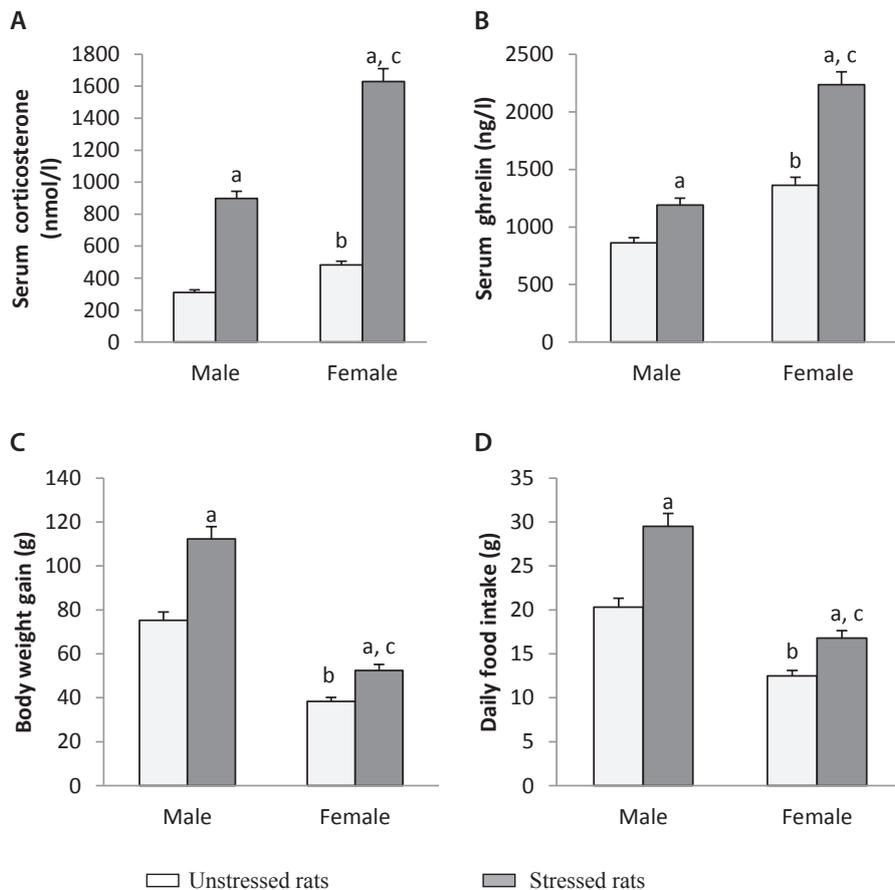
#### Results

Serum ghrelin and serum corticosterone were significantly higher in stressed vs. unstressed rats of all four groups ( $p < 0.05$ ). However, the ghrelin response to stress significantly more pronounced in sham females vs. sham males ( $p < 0.05$ ).

**Table 1.** Effect of immobilization stress on serum corticosterone, serum ghrelin, daily food intake, and body weight gain in male and female rats

	M		F		Ovx		Ovx+E	
	Unstressed	Stressed	Unstressed	Stressed	Unstressed	Stressed	Unstressed	Stressed
Serum corticosterone (nmol/l)	311.5 $\pm$ 18.4	898.2 $\pm$ 67.3 (+188.3%)	482.6 $\pm$ 43.8	1628.9 $\pm$ 73.4 (+237.5%)	368.2 $\pm$ 20.3	1117.6 $\pm$ 82.2 (+203.5%)	578.6 $\pm$ 35.2	2191.7 $\pm$ 112.8 (+278.8%)
Serum ghrelin (ng/l)	862.9 $\pm$ 97.8	1189.4 $\pm$ 113.3 (+37.8%)	1362.6 $\pm$ 92.8	2234.5 $\pm$ 152.7 (+64%)	972.8 $\pm$ 107.4	1466.2 $\pm$ 103.4 (+50.7%)	1420.5 $\pm$ 87.2	2446.9 $\pm$ 185.2 (+72.3%)
Daily food intake (g)	20.3 $\pm$ 0.6	29.5 $\pm$ 0.5 (+45.3%)	12.5 $\pm$ 0.8	16.8 $\pm$ 0.4 (+34.4%)	18.3 $\pm$ 0.3	25.9 $\pm$ 0.5 (+41.5%)	11.1 $\pm$ 0.2	14.9 $\pm$ 0.3 (+34.2%)
Body weight gain (g)	75.3 $\pm$ 7.4	112.3 $\pm$ 8.4 (+49.1%)	38.3 $\pm$ 5.3	52.5 $\pm$ 6.3 (+37.1%)	52.3 $\pm$ 4.2	75.5 $\pm$ 9.3 (+44.4%)	28.4 $\pm$ 4.3	37.9 $\pm$ 4.9 (+33.5%)

The results are expressed as the mean  $\pm$  SEM of 12 rats/group. Data in brackets are percentage change from unstressed rats. M, sham operated males with vehicle supplementation; F, sham operated females with vehicle supplementation; Ovx, ovariectomized females with vehicle supplementation; Ovx+E, ovariectomized females with estradiol benzoate supplementation;  $p < 0.05$  unstressed vs. stressed rats for all experimental groups.



**Figure 1.** Gender difference in serum corticosterone, serum ghrelin, daily food intake, and body weight gain in stressed and unstressed rats. In stressed and unstressed rats, serum corticosterone and serum ghrelin were significantly higher in the sham female group as compared to the sham male group. Daily food intake and body weight gain were significantly lower in the sham female group as compared to the sham male group. In stressed and unstressed rats, serum corticosterone and serum ghrelin were significantly higher in the sham female group as compared to the sham male group. Daily food intake and body weight gain were significantly lower in the sham female group as compared to the sham male group. The results are expressed as the mean of 12 rats. <sup>a</sup> statistical significance from unstressed rats of the same group ( $p < 0.05$ ); <sup>b</sup> statistical significance from unstressed male rats ( $p < 0.05$ ); <sup>c</sup> statistical significance from stressed male rats ( $p < 0.05$ ).

Moreover, the ghrelin response to stress was significantly less pronounced in ovariectomized females vs. sham females or ovariectomized females with estradiol benzoate supplementation ( $p < 0.05$ ). Total food intake and body weight were significantly higher in stressed vs. unstressed rats of all four groups ( $p < 0.05$ ) (Table 1).

Sham females, stressed and unstressed, displayed significant higher ghrelin and corticosterone levels than did

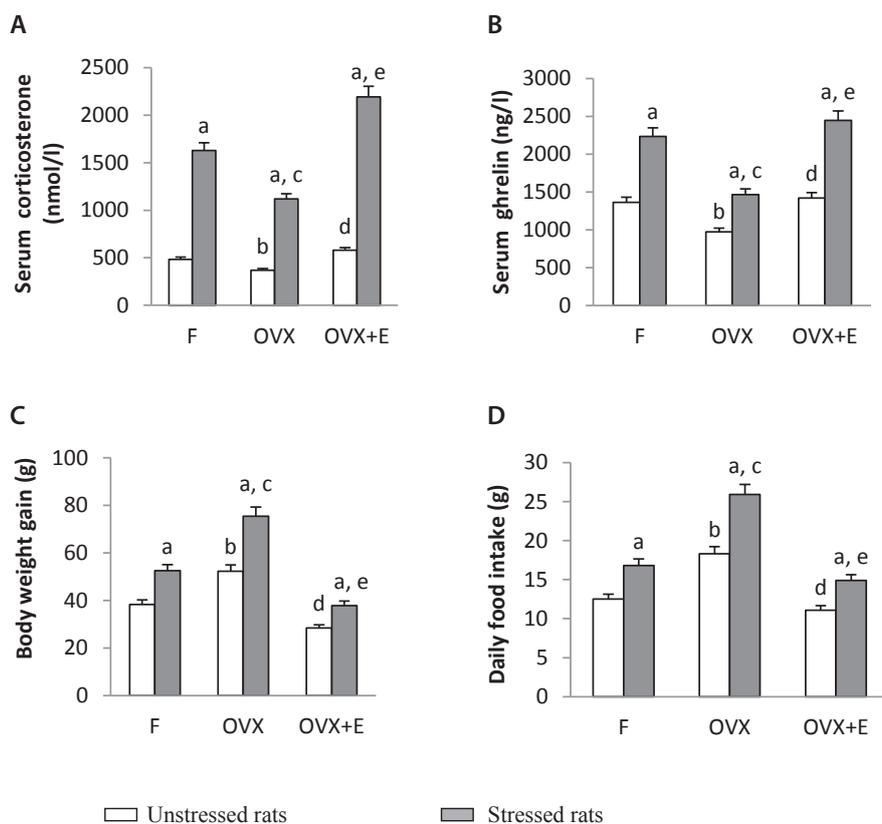
shame males, stressed and unstressed, respectively ( $p < 0.05$ ). However, daily food intake, and body weight gain were significantly higher in sham males vs. sham females ( $p < 0.05$ ) (Figure 1, Tables 2, 3).

In females, stressed and unstressed, ghrelin and corticosterone levels were significantly lower in ovariectomized females vs. sham females, this effect was reversed by estradiol benzoate supplementation ( $p < 0.05$ ).

**Table 2.** Gender difference and the effect of ovariectomy (with and without) supplementation with estradiol benzoate hormone on serum corticosterone, serum ghrelin, daily food intake, and body weight gain in unstressed male and female rats

	Unstressed			
	M	F	Ovx	Ovx+E
Serum corticosterone (nmol/l)	311.5 ± 18.4	482.6 ± 43.8 <sup>a</sup>	368.2 ± 20.3 <sup>b</sup>	578.6 ± 35.2 <sup>c</sup>
Serum ghrelin (ng/l)	862.9 ± 97.8	1362.6 ± 92.8 <sup>a</sup>	972.8 ± 107.4 <sup>b</sup>	1420.5 ± 87.2 <sup>c</sup>
Daily food intake (g)	20.3 ± 0.6	12.5 ± 0.8 <sup>a</sup>	18.3 ± 0.3 <sup>b</sup>	11.1 ± 0.2 <sup>c</sup>
Body weight gain (g)	75.3 ± 7.4	38.3 ± 5.3 <sup>a</sup>	52.3 ± 4.2 <sup>b</sup>	28.4 ± 4.3 <sup>c</sup>

The results are expressed as the mean ± SEM of 12 rats/group. M, sham operated males with vehicle supplementation; F, sham operated females with vehicle supplementation; Ovx, ovariectomized females with vehicle supplementation; Ovx+E, ovariectomized females with estradiol benzoate supplementation; <sup>a</sup> statistical significance from unstressed male rats ( $p < 0.05$ ); <sup>b</sup> statistical significance from unstressed female rats ( $p < 0.05$ ); <sup>c</sup> statistical significance from unstressed ovariectomized female rats ( $p < 0.05$ ).



**Figure 2.** Effect of ovariectomy (with and without) with estradiol benzoate hormone on serum corticosterone, serum ghrelin, daily food intake, and body weight gain in stressed and unstressed female rats: In stressed and unstressed rats, serum corticosterone and serum ghrelin significantly decreased in the OVX group as compared to sham operated group. However, estradiol benzoate supplementation to OVX rats reversed the effect of OVX on serum corticosterone and serum ghrelin and returned them to the normal levels. Daily food intake and body weight gain significantly increased in the OVX group as compared to sham operated group. However, estradiol benzoate supplementation to OVX rats reversed the effect of OVX on daily food intake and body weight gain and returned them to the normal levels. The results are expressed as the mean of 12 rats. F, sham operated females with vehicle supplementation; OVX, ovariectomized females with vehicle supplementation;

OVX+E, ovariectomized females with estradiol benzoate supplementation. <sup>a</sup> statistical significance from unstressed rats of the same group ( $p < 0.05$ ); <sup>b</sup> statistical significance from unstressed female rats ( $p < 0.05$ ); <sup>c</sup> statistical significance from stressed female rats ( $p < 0.05$ ); <sup>d</sup> statistical significance from unstressed ovariectomized female rats ( $p < 0.05$ ); <sup>e</sup> statistical significance from ovariectomized stressed female rats ( $p < 0.05$ ).

However, daily food intake, and body weight gain were significantly higher in ovariectomized females vs. sham females, this effect was reversed by estradiol benzoate supplementation to ovariectomized females ( $p < 0.05$ ) (Figure 2, Tables 2, 3).

## Discussion

The present study investigated the effect of chronic immobilization stress on serum ghrelin level in male and female rats. Serum corticosterone level was measured to confirm

**Table 3.** Gender difference and the effect of ovariectomy (with and without) supplementation with estradiol benzoate hormone on serum corticosterone, serum ghrelin, daily food intake, and body weight gain in stressed male and female rats:

	Stressed			
	M	F	Ovx	Ovx+E
Serum corticosterone (nmol/l)	898.2 ± 67.3	1628.9 ± 73.4 <sup>a</sup>	1117.6 ± 82.2 <sup>b</sup>	2191.7 ± 112.8 <sup>c</sup>
Serum ghrelin (ng/l)	1189.4 ± 113.3	2234.5 ± 152.7 <sup>a</sup>	1466.2 ± 103.4 <sup>b</sup>	2446.9 ± 185.2 <sup>c</sup>
Daily food intake (g)	29.5 ± 0.5	16.8 ± 0.4 <sup>a</sup>	25.9 ± 0.5 <sup>b</sup>	14.9 ± 0.3 <sup>c</sup>
Body weight gain (g)	112.3 ± 8.4	52.5 ± 6.3 <sup>a</sup>	75.5 ± 9.3 <sup>b</sup>	37.9 ± 4.9 <sup>c</sup>

The results are expressed as the mean ± SEM of 12 rats/group. M, sham operated males with vehicle supplementation; F, sham operated females with vehicle supplementation; Ovx, ovariectomized females, Ovx+E: ovariectomized females with estradiol benzoate supplementation; <sup>a</sup> statistical significance from stressed male rats ( $p < 0.05$ ); <sup>b</sup> statistical significance from stressed female rats ( $p < 0.05$ ); <sup>c</sup> statistical significance from stressed ovariectomized female rats ( $p < 0.05$ ).

stress exposure. In all groups, corticosterone was significantly higher among rats exposed to stress compared to unstressed rats. This increase was significantly greater in sham females compared to both sham males and ovariectomized females. Moreover, the corticosterone increase after stress exposure was significantly greater in ovariectomized females with estradiol benzoate supplementation than in ovariectomized females. These findings agree with previous results as Seale et al. (2004) who found that, following immune stress, all components of the HPA axis are activated by estrogen in females. Additionally, some other studies found that the activity of the HPA axis is markedly influenced by sex steroids, as illustrated by the pronounced elevations in glucocorticoid levels exhibited in female rodents compared with male counterparts (Pollard et al. 1975). Viau and Meaney (1991) found that in ovariectomized female rats, estradiol (E2) potentiates the corticosterone response to numerous stressors, including restraint. Additionally, because E2-dependent glucocorticoid hypersecretion to a certain extent parallels elevation in ACTH, it is believed that estrogen acts centrally to modulate the neuroendocrine responses to stress (Carey et al. 1995).

We found also that serum ghrelin was significantly higher in stressed *vs.* unstressed rats of all groups. These results are in line with some previous studies. Early on, the acute metabolic stress of fasting was established to increase gastric ghrelin mRNA expression in mice (Xu et al. 2009) and rats (Kim et al. 2003). This was associated with increased ghrelin secretion resulting in elevated circulating ghrelin levels and reversal of all these changes by feeding (Toshinai et al. 2001). The mechanisms that drive the fasting-induced increased ghrelin synthesis and inhibition upon re-feeding may be linked to changes in insulin status under these conditions as shown by the inverse correlation between changes in circulating levels of insulin and those of circulating and gastric ghrelin and ghrelin changes in response to insulin (Wang et al. 2006). Moreover, exposure to prolonged stressors such as daily 90-min restraint stress for 5 days in rats (Zheng et al. 2009) or tail pinch stress (10 min every 4 h for 24 h) in fasted mice (Asakawa et al. 2001) also increased gastric ghrelin mRNA expression (Zheng et al. 2009). Moreover, 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.

These data clearly indicate that repeated and sustained stressors such as fasting, restraint and tail pinch are associated with up-regulation of ghrelin synthesis in the rodent stomach which may account for the elevated circulating ghrelin levels under these stress conditions. Additionally, Rouach et al. (2007) study has shown for the first time that a psychological stress may induce an increase in plasma ghrelin levels in humans.

The mechanism of elevations in circulating levels of ghrelin after stress has not yet been elucidated. Speculatively, this stress-induced elevation may involve stimulation of  $\beta$ 1-adrenergic receptors on ghrelin cells, as such a pathway has been shown to play a role in ghrelin release during a 24-hour fast and as increased sympathoadrenal tone is a known consequence of stress (Sgoifo et al. 1999; Mundinger et al. 2006; Zhao et al. 2010). Or, epinephrine, which increases with stress, can increase circulating ghrelin levels (de la Cour et al. 2007).

Immunohistochemical analyses indicate that ghrelin containing neurons are found in the arcuate nucleus of the hypothalamus (ARC), a region involved in appetite regulation (Lu et al. 2002). In the ARC, these ghrelin-containing neurons send efferent fibers onto neuropeptide Y (NPY) neurons and agouti-related peptide (AgRP)-expressing neurons to stimulate the release of these orexigenic peptides, and onto pro-opiomelanocortin (POMC) neurons to suppress the release of this anorexigenic peptide, which ultimately stimulates feeding and decreases energy expenditure (Kojima et al. 1999). It was shown that ghrelin also increases food intake *via* vagal afferent-mediated neural transmission to NPY neurons in ARC (Date et al. 2002).

Under the orexigenic effect of ghrelin, the increase in serum ghrelin levels with stress exposure in this study were associated with an increase in daily food intake and body weight gain but with different extents according to each group, and this will be discussed later in the discussion.

The results of this study confirmed that there is gender differences in serum ghrelin levels either in stressed or unstressed rats. Our observation that female rats had higher levels of serum ghrelin levels than male rats are in agreement with some previous experimental reports (Greenman et al. 2004; Salbe et al. 2004) but contradict others where no gender differences were found (Tschop et al. 2001; Shiiya et al. 2002; Purnell et al. 2003; Vendrell et al. 2004; Vilarrasa et al. 2005; Weiss et al. 2006). Moreover, gender differences in ghrelin values have been described in normal subjects. It has been shown that ghrelin secretion is sexually dimorphic in humans, with women in the late follicular stage having higher levels than men (Barkan et al. 2003; Greenman et al. 2004) and that short-term change of circulating sex hormones is able to modify ghrelin levels (Gambineri et al. 2005).

Gender difference in ghrelin level can be referred to the sex hormone estrogen, since we found that ovariectomy significantly decrease serum ghrelin level and this effect is reversed by estradiol benzoate supplementation of ovariectomized rats. Previous animal studies have also shown that estrogen is involved in the regulation of ghrelin secretion and directly induced ghrelin gene expression (Matsubara et al. 2004). Ovariectomy induced a reduction in the number of ghrelin-producing cells, ghrelin mRNA levels in gastric cells,

and plasma ghrelin levels in rats. Administration of estradiol was able to reverse these changes (Matsubara et al. 2004).

Similar findings were reported in humans. Estrogen replacement therapy in 64 hysterectomized post-menopausal women receiving estrogen therapy for 6 months increased active plasma ghrelin, and the relative changes in the levels of this hormone were positively associated with the relative changes in serum estradiol concentrations (Kellokoski et al. 2005).

In this study, we found that sex interacts with stress and the ghrelin response to stress were significantly more pronounced in sham females than in sham males. This difference is likely to be estrogen-dependent because in females ghrelin response to stress was more pronounced in sham females than in ovariectomized females, and in ovariectomized females with estradiol benzoate supplementation than in ovariectomized females.

Although our results show that serum ghrelin levels were significantly lower in sham males vs. sham females, and in ovariectomized females vs. sham female or ovariectomized females with estradiol benzoate supplementation, we found that daily food intake and body weight gain were significantly higher in sham males than in sham females, and in ovariectomized females than in sham females or ovariectomized females with estradiol benzoate supplementation. This can be explained by the results of Clegg et al. (2007) study, who found that male and ovariectomized female rats were significantly more sensitive than intact female rats to the orexigenic effects of both centrally and systemically administered ghrelin. This difference referred to the female sex hormone, estrogen, because estradiol attenuated the orexigenic action of ghrelin in ovariectomized female and male rats. This means that, although estrogen increase ghrelin level but it decreases its orexigenic action. Furthermore, ovariectomy is known to induce secondary effects of hyperphagia, increased weight gain, and adiposity (McElroy and Wade 1987) as estrogen regulates food intake via anorexigenic pathways of the central nervous system (Toth et al. 2001; Asarian and Geary 2002; Liang et al. 2002).

Silva et al. (2010) and Pelletier et al. (2007) showed that estradiol treatment of Ovx rats and mice induced lower food intake and less body weight gain by reducing NPY and AgRP mRNA expression in the ARC of female rats and mice. It is also important to point out that estradiol may also interact with the action of ghrelin on NPY neurons by attenuating the orexigenic effects of this peptide produced by the stomach (Butera 2010).

There are many other mechanisms that can explain estrogen effect on food intake and body weight; Eckel et al. (2002) reported that estradiol injection increases the central processing of the vagal cholecystokinin (CCK) satiety signal in ovariectomized rats. CCK is a peptide released from the small intestine during meals and binds to receptors

on vagal afferents of the pylorus and proximal duodenum to initiate a negative-feedback satiety signal. Estrogen effectively enhances the satiety potency of CCK, leading to reductions in meal size and overall food intake (Eckel et al. 2002). Similarly, estrogen is also thought to exert inhibitory effects on feeding by augmenting glucagon-mediated satiety signaling (Geary and Asarian 2001). Additionally, the complex interaction between estrogen and leptin in the central nervous system and peripheral tissues also functions to control food intake, body weight, and adiposity (Chen and Heiman 2001; Shimomura et al. 2002; Torto et al. 2006; Gao et al. 2007).

Conclusion: To our knowledge, this is the first study showing a clear sex difference in ghrelin response to stress which is caused by estrogen, since estradiol benzoate amplifies ghrelin response to stress in females.

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