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Stress increased ghrelin secretion from pancreatic isolated islets in male rats

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Abstract. It has been demonstrated that plasma ghrelin is likely affected by stress, but little attention has been paid to the effect of stress on ghrelin release from pancreatic islets. This study investigates the effect of stress on ghrelin secretion from pancreatic islets in rats. Male Wistar rats were divided into control and stressed groups. The stressed group was further divided into foot-shock and psychological stress subgroups. Stress was induced by a communication box. After stress exposure, blood sampling was performed to determine the plasma levels of corticosterone, glucose, and ghrelin. Then the animals' pancreatic islets were isolated to assess their ghrelin output at 5.6, 8.3, and 16.7 mM glucose concentrations. Acute exposure to foot-shock and psychological stress both increased plasma corticosterone concentration. Moreover, plasma glucose concentration increased in the foot-shock stress group. Chronic exposure to foot-shock decreased plasma ghrelin concentration, whereas acute exposure had no significant effect. Acute and chronic exposure to foot-shock and psychological stress increased ghrelin secretion from isolated islets in the presence of different glucose concentrations. The results of the present study suggest that ghrelin secretion from isolated islets is not glucose-dependent. However, ghrelin secretion appears to be intensely responsive to both acute and chronic stress.

Key words: Ghrelin — Isolated islets — Corticosterone — Glucose — Stress

Introduction

Ghrelin is a 28-amino acid peptide hormone, which controls growth hormone (GH) secretion, cortisol plasma level, appetite and body weight, adiposity, gastric acid secretion, and intestinal motility (Kojima et al. 1999; Kojima and Kangawa 2005; Kristenssson et al. 2006; Rouach et al. 2007).

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Ghrelin was first derived from human and rat stomach. Ghrelin can also be isolated from many other tissues including the endocrine pancreas (Granata et al. 2007). Epsilon pancreatic cells produce and secrete ghrelin.

It is postulated that ghrelin derived from the pancreas plays an autocrine/paracrine role in regulating the survival and function of pancreatic islet beta cells. In the embryonic and neonatal periods, pancreatic ghrelin production is far greater than stomach ghrelin production. However, in adulthood, ghrelin production in the stomach dominates. This represents the potential role of ghrelin in pancreatic islet development (Wierup et al. 2004; Andralojc et al. 2009). Studies have shown that ghrelin is involved in cellular growth,

differentiation, and proliferation. It can also regenerate beta cells of the pancreas in a diabetes model in rats (Irako et al. 2006). Granata et al. (2007) also showed that acylated and unacylated ghrelin can induce proliferation of islet beta cells (HIT-T15 β cells) and prevent apoptosis of these cells and human islets of Langerhans. In this regard, studies have shown that ghrelin inhibits the expression and production of inflammatory cytokines, such as TNFα and IL1β, which are important in beta cell death. Ghrelin also inhibits the activation of caspase 3 (Granata et al. 2007).

Stress is one of the factors that has been shown to increase oxidizing agents, and it has been shown that prednisolone can increase apoptotic markers in various tissues including islets of Langerhans (Linssen et al. 2011).

Dynamics of beta cells are also affected by stress hormones and, at first, glucocorticoids (Lansang and Hustak 2011). Glucocorticoids may increase glucose and free fatty acids (FFAs) in various tissues, including the islets and beta cells, resulting in production of oxidizing agents, oxidative damage and endoplasmic reticulum (ER) stress. Glucocorticoids thereby decrease the survival of beta cells, decrease insulin synthesis, and disrupt insulin signaling pathways (Kahn 2001). Regarding relatively weak antioxidant activity in islets of Langerhans, it seems that the islets are more susceptible to stress injury (Robertson and Harmon 2006). It should be mentioned that, in addition to the indirect effects, glucocorticoids directly affect both production and secretion of insulin. In the long term, they may impair beta cell function (Beaudry and Riddell 2012). On the other hand, studies have shown that stress can either increase or decrease the plasma ghrelin concentration. For example, acute water avoidance stress in female rats increased plasma ghrelin level (Kristenssson et al. 2006), but acute novelty stress in male mice decreased it (Saegusa et al. 2011). It is noteworthy, though, that plasma ghrelin level alteration following stress is predominantly due to fluctuations in gastrointestinal ghrelin secretion. Gastrectomy has been shown to decrease plasma ghrelin concentration by 65–90% (Kristenssson et al. 2006). However, in adult human after gastrectomy 35% of circulating ghrelin remains but in adult rats the corresponding figure is about 20% (Wierup et al. 2004).

Thus it seems that ghrelin secreted by other tissues acts predominantly locally rather than systemically. Therefore, the analysis of ghrelin alteration in other tissues in response to stress is important. Since ghrelin plays a key protective role in the survival of pancreatic islet cells (Granata et al. 2007) and pancreatic islet cells play a central role in regulation of the body's metabolic activity, studying the level of ghrelin secretion in response to stress in the islets of Langerhans will be particularly important. We study here the level of ghrelin secretion from isolated pancreatic islets of Langerhans in male rats exposed to electrical foot-shock and psychological stress acutely and chronically. This study can help elucidate the pathogenesis of type II diabetes, which can arise from alteration of beta cell function subsequent to stress exposure (Robertson and Harmon 2006).

Materials and Methods

Animals

Thirty adult male Wistar rats weighing 170–190 g aged 8–10 weeks (Pasteur Institute, Tehran, Iran) were housed 3 *per* cage in a temperature controlled room (22 ± 2°C) with 12 h light/dark cycle (light on at 7:00 a.m.). Standard food (Pars Company of animal food producer, Iran) and tap water were provided throughout the experiment *ad libitum*. All animals were allowed 1 week to habituate with the environment. The animals were divided into stressed and control groups. The animals of the stressed group were further divided into foot-shock and psychological stress. The assessments of the blood parameters were done before (BB, basal before stress exposure) and after (After, immediately after stress exposure) and also one day after (BA, basal after stress exposure) acute (1 day) or chronic (15 days) exposure to stress or removing from the stress apparatus without stress exposure (for the control group) (n = 10/group). For examining the effect of acute stress on ghrelin secretion from the isolated islets, 4/group of the study animals were decapitated one day after the first exposure to stress or removing from the stress apparatus without stress exposure (for the control group). At the end of the experiment (day 16) to assess the effect of chronic stress on ghrelin secretion from the isolated islets, 4/group of the animals were decapitated and the remainder were euthanized by CO_2. All procedures were approved by the animal care and use committee of the Shahid Beheshti University of Medical Sciences, Neuroscience Research Center.

Body weight measurement

Animals' body weight was measured on the first and last (day 15) days of the experiments by a digital scale (FEW, Japan, sensitivity 0.1 g).

Food intake measurement

Food intake was measured on the 2nd, 7th and 15th days of the experiment by measuring the difference between the amount of food put in the cage and the remaining amount after 24 h.

Stress procedure

A communication box (Endo et al. 2001) (48×48×50 cm) was used as the stressor apparatus, in brief, the box was

equipped with a grid floor composed of 0.5 cm diameter stainless steel rods placed 1.3 cm apart. This device consisted of 9 chambers (16×16 cm) divided by transparent Plexiglass sheets. The grid floors of four compartments were covered by Plexiglass plate to prevent animals from receiving electric shock; thus the device has five foot-shocked and four non foot-shocked chambers. An electric shock generator (Borje Sanat, Iran) was used to produce foot-shock (1 mA, 1 Hz) for 10 s duration every 60 s (Endo et al. 2001). Animals of the psychological stress group were placed into non foot-shocked chambers in order to be exposed to various emotional stimuli (jumping, struggling, vocalizing, defecating and urinating) from the rats in the five foot-shocked chambers. Exposure to stress lasted 1 h and was performed between 10 h to 13 h. The animals of the control group were placed in the box (1 h/d) without receiving any stress. The time between the morning blood sampling and stress exposure was 2 to 5 h.

Moreover, in order to omit the effect of a novel environment, all groups of rats were placed in the communication box (1 h/day) for five days before the experiment commenced.

Blood sampling

To obtain the blood samples, animals were briefly anesthetized with isoflurane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether, Nicholas Pirmal, UK), and the samples were collected by orbital sinus puncture with a heparinized capillary micro tube (Zardooz et al. 2010), blood was collected into an Eppendorf tube containing 5 µl/ml heparin (5000 IU/ml) (Chalkley et al. 2002) and immediately centrifuged at 3000 × *g*, plasma separated and kept at –70°C for measuring the plasma parameters.

Evaluation of the plasma corticosterone, glucose and ghrelin levels

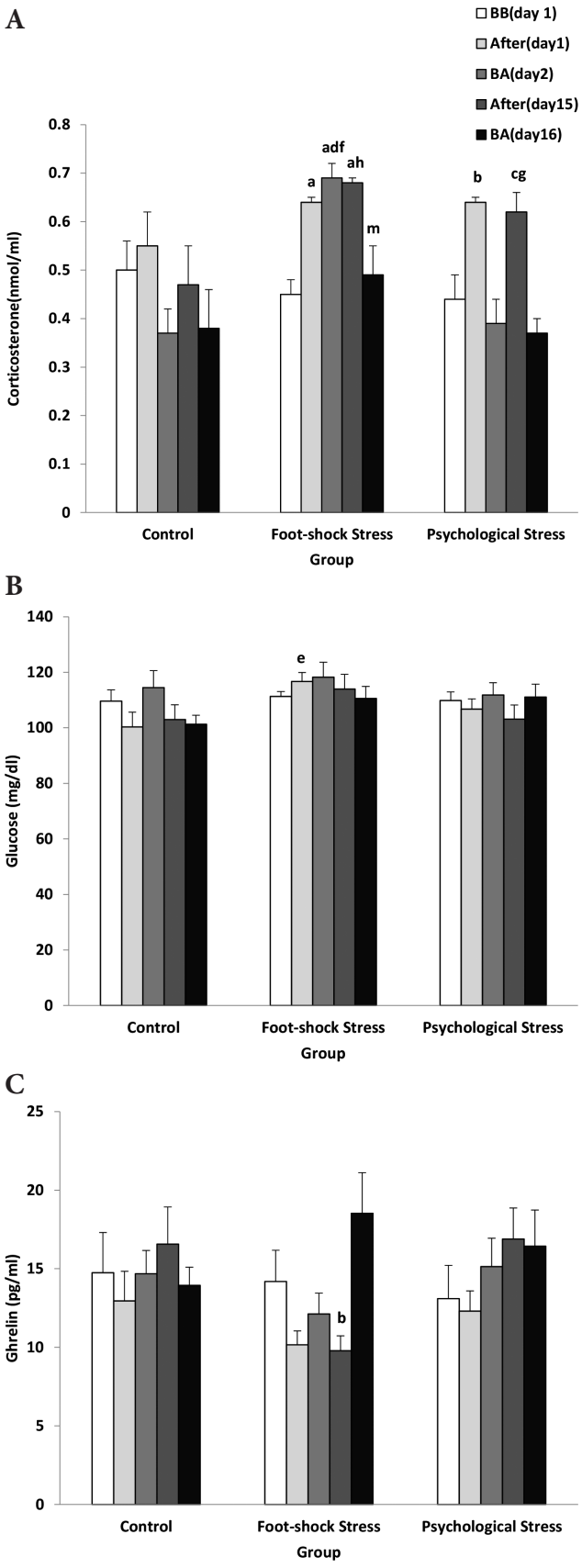
On the 1st day of the experiment, blood samples were collected from retro orbital plexus of all animals (8–8:30 a.m.) to evaluate the basal plasma corticosterone, glucose and ghrelin levels (i.e. BB). After 3 to 5 h they were placed in the communication box for 1 h without any stress application (control group) or with foot-shock or psychological stress exposure (stressed groups). The second blood sampling was performed immediately after removing the rats from the communication box (i.e. After). One hour after blood sampling they were transported to the colony room and on the next day blood sampling were performed in the rats of both control and stressed groups to evaluate the possible changes of basal plasma (i.e. BA) corticosterone, glucose and ghrelin levels due to acute exposure to stress. Following 15 days of placing the animals in the communication box (1 h/day) the blood samples were taken on the 15th day of the experiment immediately after removing the rats from the communication box (i.e. After) and in the morning of the next day of the experiment (i.e. BA) to evaluate the possible changes of basal plasma corticosterone, glucose and ghrelin levels due to chronic exposure to stress.

Islet isolation procedure

The islet isolation was performed by the collagenase technique of Lacy and Kostianovsky (1967) with slight modification. One day after the first and last stress exposure, rats were anaesthetized lightly with isoflurane, decapitated and bled (n = 4/group) and the abdomen was opened. The entrance of common bile duct to duodenum was clamped, the duct was cannulated with a polyethylene catheter (Portex Intravenous Cannula 2.5 F, 0.75 mm OD) and 10 ml cold Hank's buffer (containing in mM: NaCl, 137; KCl, 5.4; $CaCl_2$, 1.2; $MgSO_4 \times 7\ H_2O$, 0.8; $Na_2HPO_4 \times 2\ H_2O$, 0.3; KH_2PO_4, 0.4; $NaHCO_3$, 4.2 (Merk, Germany)) (Ghalami et al. 2013), in which collagenase P (Roche, Cat. # 11 213 865 001, Germany, 0.45 mg/ml) was diluted, and gently perfused into the duct. The inflated pancreas was removed and placed into a Petri dish and cleaned from non-pancreatic tissue. Then the pancreas was placed into a 50 ml falcon tube and incubated for 17 min at 37°C in water bath. Digestion was terminated by adding cold Hank's solution up to 40 ml. The tube was shaken and the suspension was dispensed into a glass container (7.5 cm diameter and 4.5 cm height). Cold Hank's solution was added and aspirated after precipitation. The supernatant of the islets was removed, a process which was repeated three times. After the last aspiration islets were hand-picked under stereomicroscope (Blue Light, USA) (first-picking).

Glucose-stimulated ghrelin secretion study

Glucose-stimulated ghrelin secretion was studied statically at 5.6, 8.3 and 16.7 mM glucose concentrations (which considered as basal (Leclercq-Meyer et al. 1991), postprandial (Nguyen et al. 2002) and pathophysiological (Chan et al. 2001) glucose concentrations in the rat plasma) to measure the ghrelin release from isolated islets in response to glucose (Lacy and Kostianovsky 1967). From the isolated islets of each animal, five groups of fifteen islets for each glucose concentrations (5.6, 8.3, 16.7 mM) were picked randomly (second-picking) and placed in the plastic cups (20 cups in to total for each condition). The average time between decapitation and placing the isolated islets in plastic cups was about 45 min. All procedures for islets separation were carried out on the ice tray. After removing the excess hank's solution, 250 µl of Krebs Ringer Solution (pH 7.4) (containing in mM: NaCl, 111; KCl, 5; $MgCl_2 \times 6\ H_2O$, 1; $CaCl_2$, 1; $NaHCO_3$, 24 (Merk, Germany); Hepes, 10 (Sigma, USA) and BSA, 0.5 g/dl (Sigma, USA)) (Ghalami et al. 2013) containing



5.6, 8.3 or 16.7 mM glucose concentrations was added to the cups and incubated for 90 min (at the beginning the cups were bubbled with 95% O_2/ 5% CO_2 for 5 min) at 37°C. Then the supernatant was removed, under a stereomicroscope to avoid collecting the islets, and stored at –70°C to evaluate its ghrelin content. These values were considered as basal after stress exposure of ghrelin secretion.

Assays

Ghrelin concentration (total ghrelin) of the supernatant and plasma was measured by Rat Ghrelin ELISA kit (Sensitivity: 1.56 pg/ml, Cusabio Biotech, China). Plasma glucose concentration was determined using the glucose oxidase method (Sensitivity: 5 mg/dl, Pars Azmoon, Iran). Corticosterone ELISA kit (Sensitivity: 1.63 nmol/l, DRG, Germany) was used to measure plasma corticosterone concentration.

Statistical analysis

All data are expressed as the mean ± SEM. A mixed analysis of variance (ANOVA) with repeated measures within the groups (time was considered as a repeated factor) and independent measures between the groups was performed and followed by Tukey test (SPSS Version 9.0 program package). Moreover two-way ANOVA tests were performed and followed by Tukey test (by considering group × glucose concentrations as factors). p value below 0.05 was considered to be statistically significant.

Results

Effects of acute (one day) and chronic (15 day) exposure to stress on plasma corticosterone concentration

Immediately after exposure to foot-shock stress on days 1 and 15, a significant increase in plasma corticosterone concentration above baseline (BB) was observed (p <

Figure 1. Plasma corticosterone (**A**), glucose (**B**) and ghrelin (**C**) concentrations before and following acute (day1) and chronic (day15) exposure to stress. Each column represents mean ± SEM for 6 rats. [a] $p < 0.001$, [b] $p < 0.01$, [c] $p < 0.05$ significant difference *vs.* "BB(day 1)" of the same group. [d] $p < 0.001$, [e] $p < 0.05$ significant difference *vs.* the control group at the same time. [f] $p < 0.001$ significant difference *vs.* the psychological group at the same time. [g] $p < 0.001$, [h] $p < 0.01$ significant difference *vs.* "BA(day 16)" of the same group. [m] $p < 0.001$ significant difference *vs.* "BA(day 2)" of the same group. BB, basal before placing inside the communication box; BA, basal after removing from the communication box; After, immediately after removing from the communication box.

0.001) (Fig. 1A). Moreover, exposure to psychological stress immediately increased plasma corticosterone level over baseline (BB) on both day 1 ($p < 0.01$) and 15 ($p < 0.05$) (Fig. 1A).

Acute exposure to foot-shock stress significantly increased basal level of the plasma corticosterone on day 2 of the experiment (BA, day 2) as compared to the control and psychological stress groups ($p < 0.001$) and also in comparison with baseline levels ($p < 0.001$) (Fig. 1A). In addition, chronic exposure to foot-shock and psychological stress (After, day 15) significantly increased plasma corticosterone concentration above baseline (BB), (($p < 0.001$) and ($p < 0.05$), respectively) (Fig. 1A) and also one day after the last stress exposure (BA, day 16) (($p < 0.01$) and ($p < 0.001$), respectively) (Fig. 1A). On the other hand, the plasma corticosterone level of the foot-shock stress group on day 16 (BA, day 16) was significantly lower than the value on day 2 (BA, day 2) ($p < 0.001$) (Fig. 1A).

Effects of acute (one day) and chronic (15 days) exposure to stress on plasma glucose concentration

As shown in Fig. 1B, acute exposure to foot-shock stress, but not psychological stress, significantly increased plasma glucose concentration only immediately after removing the animals from the communication box (After, day 2) as compared to the control group ($p < 0.05$). On the other hand, chronic exposure to foot-shock or psychological stress had no significant effect on plasma glucose concentration at any time (i.e., days 15 and 16) (Fig. 1B).

Effects of acute (one day) and chronic (15 days) exposure to stress on plasma ghrelin concentration

Immediately after exposure to foot-shock stress (day 15), plasma ghrelin concentration decreased markedly as compared to before stress exposure (BB) ($p < 0.01$), whereas at the other times the parameter did not change significantly. On the other hand, exposure to psychological stress did not affect the plasma concentration of ghrelin at any time of the experiment (Fig. 1C).

Body weight changes during the experimental period

The body weight of the control and psychological stress groups showed a significant increase on day 15 of the experiment as compared to day 1 ($p < 0.05$) (Table 1). The foot-shock stress rats did not show any marked change in body weight on day 15; however, they were significantly lighter than rats in the control and psychological stress groups on day 15 ($p < 0.05$) (Table 1).

The effect of foot-shock and psychological stress on food intake during the experimental period

The animals of the foot-shock stress group, on day 7, showed a lower food intake as compared to the animals of the other experimental groups ($p < 0.001$). Food intake on day 15 was also significantly lower than the animals of psychological stress group ($p < 0.05$) (Fig. 2).

The effect of acute (one day) and chronic (15 days) stress on ghrelin release from isolated islets

Ghrelin output from isolated islets of the acute foot-shock stress group was significantly higher than the control group, at 5.6 mM ($p < 0.05$), 8.3 mM, and 16.7 mM glucose con-

Table 1. Body weight changes in the control and stressed groups

Group	Day	
	1	15
Control	177.63 ± 5.57	196.20 ± 5.52[a]
Foot-shock stress	170.77 ± 3.91	177.42 ± 3.13[b]
Psychological stress	176.48 ± 2.50	196.40 ± 3.75[a]

[a] $p < 0.05$ significant difference *vs.* day 1of the same group; [b] $p < 0.05$ significant difference *vs.* the control and psychological groups on the same day.

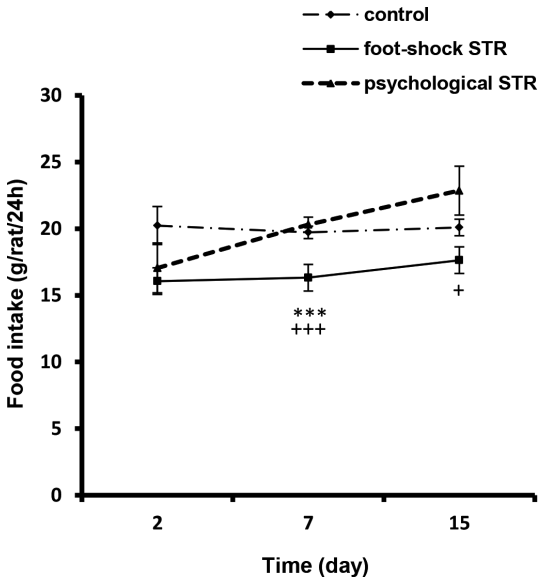


Figure 2. The effect of foot-shock and psychological stress on food intake during the experimental period. Each point represents mean ± SEM for 6 rats. *** $p < 0.001$ significant difference *vs.* the control group on the same day. $^{+++}$ $p < 0.001$, $^{+}$ $p < 0.05$ significant difference *vs.* psychological stress group on the same day. STR, stress.

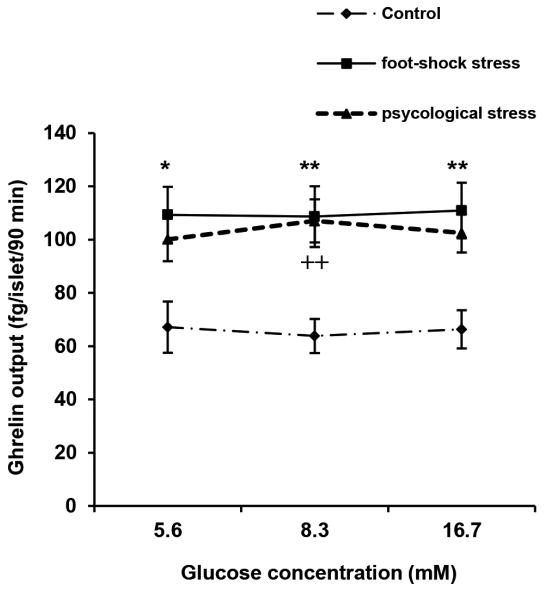


Figure 3. The effect of acute foot-shock and psychological stress on ghrelin secretion from pancreatic isolated islets. Each point represents mean ± SEM for 4 rats. ** $p < 0.01$, * $p < 0.05$ significant difference between control and foot-shock stress groups at the same glucose concentrations. $^{++}$ $p < 0.01$ significant difference between control and psychological stress groups at the same glucose concentration. STR, stress.

centrations ($p < 0.01$) (Fig. 3). In the psychological stress group, acute stress significantly increased ghrelin output from isolated islets only at the 8.3 mM glucose concentration ($p < 0.01$) as compared to the control group (Fig. 3).

Moreover, in both chronically stressed groups ghrelin output from isolated islets significantly increased over controls at 5.6 mM and 8.3 mM glucose concentrations, ($p < 0.01$ for 5.6 mM and $p < 0.001$ for 8.3 mM in foot-shock stress group; $p < 0.05$ for 5.6 mM and $p < 0.001$ for 8.3 mM in psychological stress group) (Fig. 4).

Discussion

Similar to previous research which applied foot shock and/or psychological stress using a communication box, we found rats exposed to both acute (Ishikawa et al. 1992) and chronic stress (Ishikawa et al. 1992, 1995) to have higher plasma corticosterone levels compared to the control group. Some other studies on rats, however, have failed to detect changes in plasma corticosterone concentrations following exposure to psychological stress (Endo et al. 2001). Such an inconsistency can be justified by differences in the rats' age or the timing and method of blood sampling. A comparison between electric shock and psychological stress in the current study revealed that the electric shock had more lasting effects on plasma corticosterone concentrations. In fact, although the plasma corticosterone concentrations of both groups significantly reduced on the 16th day (compared to the second day), during the days after exposure to stress, corticosterone levels were stably higher in the animals receiving foot shock. This difference in stress recovery rate can be attributed to dissimilar severity of the applied stressors (De Boer et al. 1990; Zardooz et al. 2006) or differences in pathways through which each type of stress affects the brain (Fóscolo et al. 2008). Moreover, plasma ghrelin levels had a decreasing trend following the application of the electric shock. No such a trend was observed after exposure to psychological stress. Likewise, in a study on male mice, Saegusa et al. (2011) reported a downward trend in ghrelin levels after exposure to acute stress (novelty). Furthermore, infusion of urocortin, a member of the corticotrophin-releasing factor (CRF) family, has been associated with reductions in plasma ghrelin levels in humans (Davis et al. 2004). In contrast, several studies have noticed higher plasma ghrelin levels after stress exposure. Increased plasma ghrelin levels were in fact detected following exposure to chronic stress caused by maternal separation in mice (Schmidt et al. 2006) and restraint stress in male rats (Zheng et al. 2009). Similarly, acute stress due to water avoidance elevated adrenocorticotropic

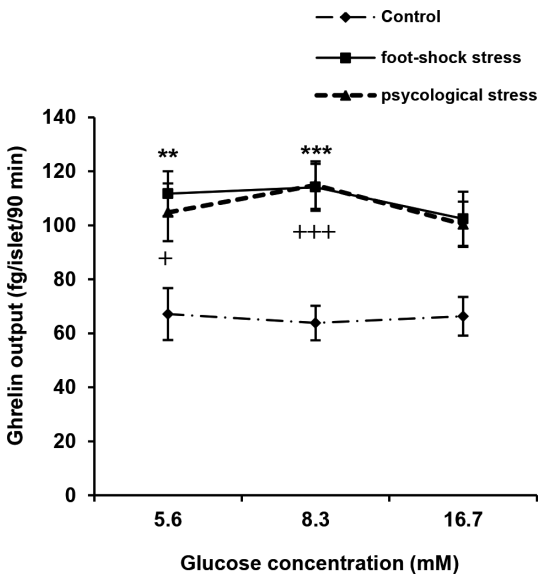


Figure 4. The effect of chronic foot-shock and psychological stress on ghrelin secretion from pancreatic isolated islets. Each point represents mean ± SEM for 4 rats. *** $p < 0.001$, ** $p < 0.01$significant difference between control and foot-shock stress groups at the same glucose concentrations. $^{+++}$ $p < 0.001$, $^{+}$ $p < 0.01$significant difference between control and psychological stress groups at the same glucose concentrations. STR, stress.

hormone (ACTH) and plasma ghrelin levels in Sprague Dawley rats (Kristenssson et al. 2006). The type of stress and the pathway each type uses to stimulate the brain might have been responsible for the differences in the obtained results. According to Saegusa et al. (2011), activation of the hypothalamic-pituitary-adrenal (HPA) axis and increment of CRF would activate CRF receptor 1 (CRF-R1) and thus a signaling pathway through which the vagus is stimulated and ghrelin secretion in the stomach is decreased. The same mechanism can explain the reduced plasma ghrelin levels in the present study. On the other hand, considering the possible presence of β1-adrenergic receptor in ghrelin secreting cells, the dominance of sympathoadrenal activity might account for increased levels of plasma ghrelin following stress exposure in other studies (Zhao et al. 2010).

Based on our findings, there was only a marginal increase in plasma glucose levels of the foot shock stress group on the second day. Similarly, in a study on male mice, Katsura et al. (2002) indicated the absence of significant changes in plasma glucose levels following electric shock and psychological stress using the communication box. Furthermore, plasma glucose levels of male non diabetic Wistar rats did not significantly change after electric shock and psychological stress induced by the communication box (Amano et al. 2007). The inverse relationship between plasma ghrelin and glucose levels, which had been previously confirmed (Erdmann et al. 2004), was not present in the current research. Therefore, elevations in HPA axis activity and plasma corticosterone levels were probably the main determinants of plasma ghrelin levels in our study. In addition, foot shock might have increased ghrelin clearance by the kidneys and hence reduced plasma ghrelin levels in the present research (Nahata et al. 2014). According to our findings, increased corticosterone and decreased ghrelin levels resulted in significantly lower food consumption and weight gain in rats exposed to electric shock compared to the other two groups. This is of course a rational finding since elevated corticosterone levels and the consequent reduction in plasma levels of ghrelin (the appetite stimulating hormone) (Wren et al. 2001) following the electric shock could naturally decrease the rats' food consumption and thus weight gain.

In the present study, both acute and chronic stress caused by either foot shock or psychological stress, increased ghrelin release from the islets. Such elevations were independent from glucose concentrations. However, plasma ghrelin levels showed a downward trend, particularly in response to foot shock stress. In other words, in contrast to the plasma ghrelin levels which generally reflect the level of ghrelin secretion in the stomach, the activity of ghrelin-releasing cells in the islets does not depend on the type of stress or glucose concentrations. While previous research has mostly focused on ghrelin secretion from gastric X/A-like cells and ghrelin secretion from the islets has not been thoroughly studied, similar mechanisms may be involved in the effects of stress on both processes. Elevations in gastric ghrelin mRNA have been documented following acute stress (tail pinch) in mice (Asakawa et al. 2001). Moreover, five days of continuous exposure to the stress caused by floating in water (2-cm deep) increased prepro-ghrelin mRNA and the number of ghrelin-secreting cells in the gastric body of Wistar rats (Ochi et al. 2008). Therefore, in the present study, the elevation in corticosterone levels induced by stress might have raised ghrelin secretion from the islets through increasing ghrelin mRNA expression or the number of ghrelin-secreting cells. Furthermore, stress can increase the plasma levels of norepinephrine and epinephrine and stimulate ghrelin secretion through these catecholamines. Both intraperitoneal (IP) and intra-paraventricular nucleus (PVN) administration of CRF have been shown to stimulate the sympathetic nervous system (Diz-Chaves 2011). The consequent release of norepinephrine and epinephrine would then increase ghrelin release (Uchida et al. 2014). Cour et al. concluded that direct administration of norepinephrine and epinephrine to the oxyntic mucosa stimulated ghrelin secretion in conscious rats (De la Cour et al. 2007). In addition, stimulation of intestinal sympathetic nerves is known to promote ghrelin secretion (Mundinger et al. 2006). Thus, it might be possible that increased sympathetic nerve activity to the pancreas, caused by stress exposure, enhanced ghrelin secretion. On the other hand, since ghrelin possesses antioxidant and anti-apoptotic (protective in general) effects (Granata. et al. 2007), the possible stress-induced elevation of inflammatory cytokines might also be involved in the stimulation of ghrelin release from the islets. These factors should, however, be further studied.

While gastric ghrelin is known to be dependent on glucose levels, we did not observe such dependence in case of ghrelin response in the islets. Therefore, ghrelin might act as a paracrine factor in the islets. Geranata et al. (2007) suggested that ghrelin played a paracrine/autocrine role to ensure the survival and performance of beta cells in the Islets of Langerhans. Since the islets' response in this study did not depend on glucose levels, basal secretion of ghrelin from the islets might have been observed.

In conclusion, the present study indicated that ghrelin secretion from the islets of Langerhans and plasma ghrelin level might have different mechanisms. The first was in fact independent from plasma glucose concentrations and was equally affected by both acute and chronic stress. Apparently, ghrelin-secreting cells in the islets are highly sensitive to stress and the consequences of increased corticosterone. They thus try to prevent the destructive effects of stress on the islets by secreting more ghrelin. The current research can shed light on mechanisms involved in the stress response in the islets of Langerhans. Considering the role of stress in the incidence of type 2 diabetes (Pouwer et al. 2010), our

findings can be beneficial in the development of methods to prevent or treat this prevalent disease.

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References

Amano M., Suemaru K., Cui R., Umeda Y., Li B., Gomita Y., Kawasaki H., Araki H. (2007): Effects of physical and psychological stress on 5-HT2A receptor-mediated wet-dog shake responses in streptozotocin-induced diabetic rats. Acta. Med. Okayama **61,** 205–212

Andralojc K. M., Mercalli A., Nowak K. W., Albarello L., Calcagno R., Luzi L., Bonifacio E., Doglioni C., Piemonti L. (2009): Ghrelin-producing epsilon cells in the developing and adult human pancreas. Diabetologia **52,** 486–493 http://dx.doi.org/10.1007/s00125-008-1238-y

Asakawa A., Inui A., Kaga T., Yuzuriha H., Nagata T., Fujimiya M., Katsuura G., Makino S., Fujino M. A., Kasuga M. (2001): A role of ghrelin in neuroendocrine and behavioral responses to stress in mice. Neuroendocrinology **74,** 143–147 http://dx.doi.org/10.1159/000054680

Beaudry J. L., Riddell M. C. (2012): Effects of glucocorticoids and exercise on pancreatic β-cell function and diabetes development. Diabetes. Metab. Res. Rev. **28,** 560–573 http://dx.doi.org/10.1002/dmrr.2310

Chalkley S. M., Hettiarachchi M., Chisholm D. J., Kraegen E. W. (2002): Long-term high-fat feeding leads to severe insulin resistance but not diabetes in Wistar rats. Am. J. Physiol. Endocrinol. Metab. **282,** 1231–1238 http://dx.doi.org/10.1152/ajpendo.00173.2001

Chan O., Chan S., Inouye K., Vranic M., Matthews S. G. (2001): Molecular regulation of the hypothalamo- pituitary-adrenal axis in streptozotocin-induced diabetes: effects of insulin treartment. Endocrinology **142,** 4872–4879 http://dx.doi.org/10.1210/endo.142.11.8474

Davis M. E., Pemberton C. J., Yandle T. G., Lainchbury J. G., Rademaker M. T., Nicholls M. G., Frampton C. M., Richards A. M. (2004): Urocortin-1 infusion in normal humans. J. Clin. Endocrinol. Metab. **89,** 1402–1409 http://dx.doi.org/10.1210/jc.2003-031231

De Boer S. F., Koopmans S. J., Slangen J. L., Van Der Gugten J. (1990): Plasma catecholamine, corticosterone and glucose responses to repeated stress in rats: effect of interstressor interval length. Physiol. Behav. **47,** 1117–1124 http://dx.doi.org/10.1016/0031-9384(90)90361-7

De la Cour C. D., Norlen P., Hakanson R. (2007): Secretion of ghrelin from rat stomach ghrelin cells in response to local microinfusion of candidate messenger compounds: a microdialysis study. Regul. Pept. **143,** 118–126 http://dx.doi.org/10.1016/j.regpep.2007.05.001

Diz-Chaves Y. (2011): Ghrelin, appetite regulation, and food reward: interaction with chronic stress. Int. J. Pept. **2011,** 898450 http://dx.doi.org/10.1155/2011/898450

Endo Y., Yamauchi K., Fueta Y., Lrie M. (2001): Changes of body temperature and plasma corticosterone level in rats during psychological stress induced by the communication box. Med. Sci. Monit. **7,** 1161–1165

Erdmann J., Töpsch R., Lippl F., Gussmann P., Schusdziarra V. (2004): Postprandial response of plasma ghrelin levels to various test meals in relation to food intake, plasma insulin, and glucose. J. Clin. Endocrinol. Metab. **89,** 3048–3054 http://dx.doi.org/10.1210/jc.2003-031610

Fóscolo D. R., Fóscolo R. B., Marubayashi U., Reis A. M., Coimbra C. C. (2008): Neonatal maternal separation affects endocrine and metabolic stress responses to ether exposure but not to restraint exposure in adult rats. Metab. Brain. Dis. **23,** 375–385 http://dx.doi.org/10.1007/s11011-008-9102-9

Ghalami J., Zardooz H., Rostamkhani F., Farrokhi B., Hedayati M. (2013): Glucose-stimulated insulin secretion: Effects of high-fat diet and acute stress. J. Endocrinol. Invest. **36,** 835–842

Granata R., Settanni F., Biancone L., Trovato L., Nano R., Bertuzzi F., Destefanis S., Annunziata M., Martinetti M., Catapano F. et al. (2007): Acylated and unacylated ghrelin promote proliferation and inhibit apoptosis of pancreatic beta-cells and human islets: involvement of 3‘,5‘-cyclic adenosine monophosphate/protein kinase A, extracellular signal-regulated kinase 1/2, and phosphatidyl inositol 3-Kinase/Akt signaling. Endocrinology **148,** 512–529 http://dx.doi.org/10.1210/en.2006-0266

Irako T., Akamizu T., Hosoda H., Iwakura H., Ariyasu H., Tojo K., Tajima N., Kangawa K. (2006): Ghrelin prevents development of diabetes at adult age in streptozotocin-treated newborn rats. Diabetologia **49,** 1264–1273 http://dx.doi.org/10.1007/s00125-006-0226-3

Ishikawa M., Hara C., Ohdo S., Ogawa N. (1992): Plasma corticosterone response of rats with sociopsychological stress in the communication box. Physiol. Behav. **52,** 475–480 http://dx.doi.org/10.1016/0031-9384(92)90333-W

Ishikawa M., Ohdo S., Watanabe H., Hara C., Ogawa N. (1995): Alteration in circadian rhythm of plasma corticosterone in rats following sociopsychological stress induced by communication box. Physiol. Behav. **57,** 41–47 http://dx.doi.org/10.1016/0031-9384(94)00192-8

Kahn S. E. (2001): The importance of beta-cell failure in the development and progression of type 2 diabetes. J. Clin. Endocrinol. Metab. **86,** 4047–4058

Katsura M., Mohri Y., Shuto K., Tsujimura A., Ukai M., Ohkuma S. (2002): Psychological stress, but not physical stress, causes increase in diazepam binding inhibitor (DBI) mRNA expression in mouse brains. Brain. Res. Mol. Brain. Res. **104,** 103–109 http://dx.doi.org/10.1016/S0169-328X(02)00219-X

Kojima M., Hosoda H., Date Y., Nakasato M., Matsuo H., Kangawa K. (1999): Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. **402,** 656–660 http://dx.doi.org/10.1038/45230

Kojima M., Kangawa K. (2005): Ghrelin: structure and function. Physiol. Rev. **85,** 495–522 http://dx.doi.org/10.1152/physrev.00012.2004

Kristenssson E., Sundqvist M., Astin M., Kjerling M., Mattsson H., Dornonville de la Cour C., Håkanson R., Lindström E. (2006):

Acute psychological stress raises plasma ghrelin in the rat. Regul. Pept. **134,** 114–117
http://dx.doi.org/10.1016/j.regpep.2006.02.003

Lacy P. E., Kostianovsky M. (1967): Method for the isolation of intact islets of langerhans from the rat pancreas. Diabetes **16,** 35–39
http://dx.doi.org/10.2337/diab.16.1.35

Lansang M. C., Hustak L. K. (2011): Glucocorticoid- induced diabetes and adrenal suppression: how to detect and manage them. Cleve. Clin. J. Med. **78,** 748–756
http://dx.doi.org/10.3949/ccjm.78a.10180

Leclercq-Meyer V., Akkan A. G., Marchand J., Malaisse W. J. (1991): Effect of glimepiride and glibenclamide on insulin and glucagon secretion by the perfused rat pancreas. Biochem. Pharmacol. **42,** 1634–1637
http://dx.doi.org/10.1016/0006-2952(91)90435-8

Linssen M. M., van Raalte D. H., Toonen E. J., Alkema W., van der Zon G. C., Dokter W. H., Diamant M., Guigas B., Ouwens D. M. (2011): Prednisolone-induced beta cell dysfunction is associated with impaired endoplasmic reticulum homeostasis in INS-1E cells. Cell Signal. **23,** 1708–1715
http://dx.doi.org/10.1016/j.cellsig.2011.06.002

Mundinger T. O., Cummings D. E., Taborsky G. J. Jr. (2006): Direct stimulation of ghrelin secretion by sympathetic nerves. Endocrinology **147,** 2893–2901
http://dx.doi.org/10.1210/en.2005-1182

Nahata M., Saegusa Y., Sadakane C., Yamada C., Nakagawa K., Okubo N., Ohnishi S., Hattori T., Sakamoto N., Takeda H. (2014): Administration of exogenous acylated ghrelin or rikkunshito, an endogenous ghrelin enhancer, improves the decrease in postprandial gastric motility in an acute restraint stress mouse model. Neurogastroenterol. Motil. **26,** 821–831
http://dx.doi.org/10.1111/nmo.12336

Nguyen Q. A., Antoine M. H., Ouedraogo R., Hermann M., Sergooris J., Pirotte B., Masereel B., Lebrun P. (2002): In vitro and in vivo effects of new insulin releasing agents. Biochem. Pharmacol. **63,** 515–521
http://dx.doi.org/10.1016/S0006-2952(01)00880-2

Ochi M., Tominaga K., Tanaka F., Tanigawa T., Shiba M., Watanabe T., Fujiwara Y., Oshitani N., Higuchi K., Arakawa T. (2008): Effect of chronic stress on gastric emptying and plasma ghrelin levels in rats. Life Sci. **82,** 862–868
http://dx.doi.org/10.1016/j.lfs.2008.01.020

Pouwer F., Kupper N., Adriaanse M. C. (2010): Does emotional stress cause type 2 diabetes mellitus? A review from the European Depression in Diabetes (EDID) Research Consortium. Discov. Med. **9,** 112–118

Robertson R. P., Harmon J. S. (2006): Diabetes, glucose toxicity, and oxidative stress: A case of double jeopardy for the pancreatic islet beta cell. Free. Radic. Biol. Med. **41,** 177–184
http://dx.doi.org/10.1016/j.freeradbiomed.2005.04.030

Rouach V., Bloch M., Rosenberg N., Gilad S., Limor R., Stern N., Greenman Y. (2007): The acute ghrelin response to a psychological stress challenge does not predict the post-stress urge to eat. Psychoneuroendocrinology **32,** 693–702
http://dx.doi.org/10.1016/j.psyneuen.2007.04.010

Saegusa Y., Takeda H., Muto S., Nakagawa K., Ohnishi S., Sadakane C., Nahata M., Hattori T., Asaka M. (2011): Decreased plasma ghrelin contributes to anorexia following novelty stress. Am. J. Physiol. Endocrinol. Metab. **301,** E685–696
http://dx.doi.org/10.1152/ajpendo.00121.2011

Schmidt M. V., Levine S., Alam S., Harbich D., Sterlemann V., Ganea K., de Kloet E. R., Holsboer F., Müller M. B. (2006): Metabolic signals modulate hypothalamic-pituitary-adrenal axis activation during maternal separation of the neonatal mouse. J. Neuroendocrinology **18,** 865–874
http://dx.doi.org/10.1111/j.1365-2826.2006.01482.x

Uchida A., Zechner J. F., Mani B. K., Park W. M., Aguirre V., Zigman J. M. (2014): Altered ghrelin secretion in mice in response to diet-induced obesity and Roux-en-Y gastric bypass. Mol. Metab. **3,** 717–730
http://dx.doi.org/10.1016/j.molmet.2014.07.009

Wierup N., Yang S., McEvilly R. J., Mulder H., Sundler F. (2004): Ghrelin is expressed in a novel endocrine cell type in developing rat islets and inhibits insulin secretion from INS-1 (832/13) cells. J. Histochem. Cytochem. **52,** 301–310
http://dx.doi.org/10.1177/002215540405200301

Wren A. M., Seal L. J., Cohen M. A., Brynes A. E., Frost G. S., Murphy K. G., Dhillo W. S., Ghatei M. A., Bloom S. R. (2001): Ghrelin enhances appetite and increases food intake in humans. J. Clin. Endocrinol. Metab. **86,** 5992
http://dx.doi.org/10.1210/jcem.86.12.8111

Zardooz H., Zahediasl S., GharibNaseri M. K. (2006): Effect of chronic psychological stress on insulin release from rat isolated pancreatic islets. Life Sci. **79,** 57–62
http://dx.doi.org/10.1016/j.lfs.2005.12.032

Zardooz H., Rostamkhani F., Zaringhalam J., Shahrivar F. F. (2010): Plasma corticosterone, insulin and glucose changes induced by brief exposure to isoflurane, diethyl ether and CO2 in male rats. Physiol. Res. **59,** 973–978

Zhao T. J., Sakata I., Li R. L., Liang G., Richardson J. A., Brown M. S., Goldstein J. L., Zigman J. M. (2010): Ghrelin secretion stimulated by {beta} 1-adrenergic receptors in cultured ghrelinoma cells and in fasted mice. Proc. Natl. Acad. Sci. USA **107,** 15868–15873
http://dx.doi.org/10.1073/pnas.1011116107

Zheng J., Dobner A., Babygirija R., Ludwig K., Takahashi T. (2009): Effects of repeated restraint stress on gastric motility in rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. **296,** R1358–1365
http://dx.doi.org/10.1152/ajpregu.90928.2008

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