THE COMPARISON OF THE EFFECTS OF CHRONIC RESTRAINT STRESS ON SERUM GHRELIN CONCENTRATION, FOOD INTAKE AND BODY WEIGHT IN NORMAL WEIGHT AND OBESE RATS

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ABSTRACT: Background: Ghrelin secretion occurs not only in response to states of energy insufficiency but also following stress. In addition, there is an association between body weight and serum ghrelin concentration. The aim of this study was the comparison of the effects of chronic restraint stress on serum ghrelin concentration, food intake and body weight in normal weight and obese rats.

Methods: Forty Sprague Dawley male rats (20 normal weight and 20 obese rats) were studied. Rats were subjected to restraint stress 2 hours per day for 14 days. Food consumption and body weight were measured daily. Serum ghrelin concentration was measured by the ELISA. Results: Restriant stress caused a significant decrease in serum of ghrelin level (120.3 ± 31.5 ng/ml vs. 936.5 ± 30 ng/ml (in normal weight rats). But stress has no significant effect on the ghrelin concentration in obese rats. The stress significantly decreased (P<0.0001) food intake in normal weight and obese rats. This stress has no significant effect on body weight in normal weight and obese rats. Conclusion: The results of this study indicate that ghrelin has a role in the eating behavior of normal weight rats and no obese rats in stress conditions.

Keywords: Stress, Ghrelin, Food intake, Body weight.

INTRODUCTION

Stress has effect on eating behavior and body weight in human and animal models [1,2,3,4,5]. Some previous studies have been shown decrease [6,1, 2) and others have been shown increase in food intake following stress [5, 7]. It seems effect of stress on food consumption dependent to adiposity and body mass index [4, 8]. For instance, it was shown that stress situations associated with overeating more strongly in the men with higher BMI [8].

Ghrelin, an appetite-stimulating hormone was originally isolated from the rat stomach [9]. Ghrelin administration stimulates GH secretion but also causes weight gain by increasing food intake and reducing fat utilization in rodents. A number of previous studies have been shown, rises in ghrelin occur not only in response to states of
energy insufficiency but also following stress [10, 11, 12]. In addition, it seems there is an association between body weight and ghrelin concentration [13, 14, 15]. Plasma ghrelin concentrations in the patients with simple obesity was lower than those of subjects with normal body weight [16].

For more investigation about effect of BMI on food consumption in stress situations and possible involvement of ghrelin in the eating behavior of normal and obese individuals following stress we measured serum ghrelin concentrations, food intake and body weight in obese stressed rats and compare it with serum ghrelin concentration, food intake and body weight in normal weight stressed rats.

MATERIALS AND METHODS

Forty male Wistar rats, body weight 150-200 g were used in the study. The animals were maintained under standard conditions (temperature 22±2°C, lights on at 6:00 to 18:00 hours). After one week of acclimatization, the rats were randomized either on a standard chow diet (n=20) or on a high-fat diet (n=20) ad libitum for 5-weeks. Fat diet was a mixture of standard chow and sunflower oil. At the end of the nutritional period, rats were fed with a high-fat diet showed significant increase in total body weight (347.2 ± 12.2g) compare with rats were fed with standard diet (214.4 ± 4.4g). During experimental period obese rats were fed with standard diet. Food consumption and body weight were measured daily. All animal experimental procedures were done in accordance with AJA University of Medical Sciences ethic committee.

Stress Procedure

Rats were subjected to restraint stress in a plexiglas restrainer tubes 2 hours per day for 14 days. The experimental period was between 10:00 and 12:00 h. On the 14th day, after stress induction, trunk blood was collected and serum samples were obtained by centrifugation of blood at 3500 for five minutes. Serum was frozen until the day of the analysis. Measurement of serum ghrelin was done with a commercial rat ghrelin ELISA kit (East Bio Pharm ).

Statistical analysis

All values were expressed as Mean ± S.E.M. Statistical analyses were performed using spss16 software. One way ANOVA analysis and Post hoc test (LSD) were used to compare mean among the groups. Associations between ghrelin concentration and body weight were assessed by liner correlation (Pearson). Differences were considered significant if P values were less than 0.05.

RESULTS

The effect of restraint stress on serum ghrelin concentration has been shown in fig1. Stress significantly decreased ghrelin concentration in normal weight rats (P=0.001). But stress had no significant effect on ghrelin concentration in obese rats.

In addition, There was no significant difference in ghrelin concentration between normal weight and obese rats in unstressed conditions but there was significant difference (P=0.004) in ghrelin concentration between normal weight and obese rats in stressed conditions (Fig1).
Figure 1: The effect of restraint stress on serum ghrelin concentration in normal weight and obese male rats. CN = unstressed normal weight rats; SN = stressed normal weight rats; CO = unstressed obese rats; SO = stressed obese rats. Data are mean ± SEM. *p = 0.001 vs. CN group. #p = 0.004 vs. CN group.

Stress significantly decreased (P<0.0001) food intake in normal weight and obese rats (Fig 2). There was significant difference in food intake between two stressed groups (Fig 2).

Figure 2: The effect of restraint stress on food intake in normal weight and obese male rats. CN = unstressed normal weight rats; SN = stressed normal weight rats; CO = unstressed obese rats; SO = stressed obese rats. Data are mean ± SEM. *p < 0.0001 vs. CN group. #P < 0.0001 vs. SN group. Ж P < 0.0001 vs. SN group.
The stress had no significant effect on body weight in normal weight and obese rats (Fig 3). There was not significant correlation between ghrelin concentration and body weight among rats in unstressed conditions ($r = -0.2$, $p=0.3$). But there was significant negative correlation between ghrelin concentration and body weight among rats in stressed conditions ($r=-0.54$, $P=0.01$).

![Figure 3: The effect of restraint stress on body weight in normal weight and obese male rats. CN= unstressed normal weight rats; SN= stressed normal weight rats; CO= unstressed obese rats; SO= stressed obese rats. Data are mean± SEM.](image)

**DISCUSSION**

In most previous animal and human studies, the effect of stress on ghrelin concentration has been measured in normal weight subjects. [6,17,18] but, present study investigated the effect of chronic restraint stress on serum ghrelin level, food intake and body weight in both groups of normal and overweight rats. Our study showed stress resulted to decrease in ghrelin concentration in normal weight rats. In this regard some of previous studies reported increase [19, 20, 17] and others reported decrease in ghrelin concentration [21,18, 22]. Due to orexigenic effect of ghrelin, it was expected, stress-induced decrease in serum ghrelin concentration resulted to decrease in food intake. In consistent with our study, a number of previous studies reported, stress resulted to decrease in food intake in normal weight rats [23, 6, 24]. The mechanisms responsible for stress-based eating behaviors mostly unknown. Chagra et al. [25] reported acute or repeated (30 min/d × 14 d) restraint stress reduce the number of agouti related peptide (AgRP) -expressing cells in hypothalamus of male rats. AgRP and neuropeptide Y (NPY) are orexigenic hypothalamic neuropeptides. Also, 2 hours after the foot-shock stress, AgRP mRNA levels in the arcuate nucleus of hypothalamus were markedly reduced [26]. The stress-induced reduction in food intake has been demonstrated both as an acute response after a single stress and as a maintained decrease in 24 h food intake during and after repeated daily restraint stress [27].Also, composition of foods affect on stress-induced food intake and stress per se, without high calorie input does not result in body weight gain. Rats submitted to foot shock stress decreased the intake of commercial chow, but kept unaltered the intake of comfort food [28]. In contrast to normal diet, when sweet food was presented to the stressed animals, repeated mild pinch resulted in hyperphagia and considerable gain in body weight [29].
In the present study, there was no significant difference in serum ghrelin concentration between normal and overweight rats in unstressed conditions. Furthermore, conflict to recent reports, we have not found an association between body weight and ghrelin concentration in unstressed conditions. This difference may be related to differences in the population studied because more previous studies investigated relation between ghrelin and BMI in human populations [13, 15, 30]. In this study, serum ghrelin concentration did not significantly change in obese rats following stress. Nevertheless, food consumption significantly decreased. There are reports that show in obesity total plasma ghrelin and its associations with food intake and stress are decreased and central resistance to ghrelin develops [31]. Consistent with our study, Nishizawa et al. [20] reported emotional stress has no significant effect on the body weight.

CONCLUSIONS
In this study, stress resulted to decrease in ghrelin concentration only in normal weight rats but stress resulted to decrease in food consumption in normal weight and obese rats. Therefore, it seems that ghrelin has a role in the eating behavior of normal weight rats and no obese rats in stress conditions. Future work will be needed to explore the role of ghrelin in the eating behavior of obese subjects in stress conditions.

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REFERENCES

15. Haq AM, Farooqi IS, O'Rahilly S, Stadler DD, Rosenfeld RG, Pratt KL, et al. Serum ghrelin levels are inversely correlated with body mass index, age, and insulin concentrations in normal children and are markedly increased in Prader-Willi syndrome. J Clin Endocrinol Metab. 2003 Jan; 88(1): 174-8.