Original Article

Effects of Isolation and Social Subchronic Stresses on Food Intake and Levels of Leptin, Ghrelin, and Glucose in Male Rats

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Abstract

Background: Exposure to psychological stresses can be a reason for obesity. Therefore, identifying the effective nutritional mechanisms such as feeding markers is of high necessity for the psychological stress conditions. Hence, the present study investigates the effects of subchronic isolation and social stresses on food intake, body weight differences (BWD), and levels of leptin, ghrelin, and glucose in rats. **Materials and Methods:** Eighteen male rats were randomly allocated into three groups: control (Co), isolation stress (IS), and social stress (SS) groups. Rats were under stresses for 7 days. The food intake (for three continuous hours after 16–18 h of food deprivation), BWD, levels of ghrelin, leptin, and glucose were measured. **Results:** The results showed that the food intake significantly ($P < 0.05$) reduced during the 1st h in the SS group compared to the Co group. At the 2nd h, the food intake significantly ($P < 0.001$ and $P < 0.01$, respectively) decreased in the IS group compared to the Co and SS groups. The cumulative food intake and body weight were significantly ($P < 0.05$) reduced in the IS group compared to the Co group. The serum ghrelin level significantly reduced in the IS group compared to the Co group. **Conclusions:** The subchronic psychological stresses led to a reduction in food intake by the reduction of serum ghrelin levels. It seems that ghrelin might have a more fundamental role in the food intake with respect to the leptin and glucose levels in subchronic stress condition. Furthermore, the decreased body weight justified the reduction of food intake, particularly in subchronic isolation stress.

Keywords: *Food intake, ghrelin, glucose, isolation stress, leptin, social stress*

Introduction

The balance between food intake and energy expenditure is regulated by the nervous system through complex mechanisms.[1] Although several studies have been conducted to determine the complex arrays of internal and external factors that affect feeding, nutritional mechanisms are still in a state of ambiguity.[2,3] In this regard, stress as an inseparable aspect of daily life has been known as a common external factor that can lead to physiological and behavioral impairments such as changes in the eating patterns.[4,5] Stress responses are composed of a variety of replies originating from central and peripheral systems.[6]

Obesity as a global concern results from physiological disturbances such as exposure to psychological stresses.[7] It seems that the effective regulatory mechanisms of nutrition and stress are really important to be identified.[7,8] An important issue in this regard is that stressful events can change the neuroendocrine signaling.[3] Various hormonal and feeding biomarkers interactions regulate the feeding behavior in stressful situations.[3] Some studies have shown that the feeding biomarkers (such as ghrelin, leptin, and glucose levels) have a key role in homeostasis and can regulate the food intake as peripheral responses.[2,9,10]

Moreover, it has been demonstrated that various types and durations of stress lead to different physiological effects.[11,12] As previous studies indicated, the type of stress plays a key role in determining the amount of food consumption.[6,13] Furthermore, the duration of stress can change the feeding patterns.[13,14] Based on the stress duration category, a variety of acute, subchronic, and chronic stress exists.[11,15-18] Acute stress suppresses the appetite as an instant physiological response.[4] While, seeking out and eating happens in the chronic stress situations.[4,19] Furthermore,

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the amount of food intake is regulated by different mechanisms under various stress conditions.[4,13,20-23] In this regard, the effect of two common types of psychological stresses (social and isolation stress) in human societies on food intake and feeding biomarkers is still unknown. Today, the most influence of stress model on human societies is subchronic stress conditions. To the best of our knowledge, there was no exact evidence of the food intake changes in subchronic psychological stresses up to now. Hence, the present study was conducted to investigate the effects of two subchronic psychological stresses (social and isolation stress) on food intake, body weight, ghrelin, leptin, and glucose levels in rats.

Materials and Methods

Experimental procedure

Animals

In this work, 18 male Wistar rats were obtained from Pasteur Institute, Tehran, Iran, with an initial body weight of 200–250 g. Rats were housed under standard laboratory conditions; on a 12 h light/dark cycle at controlled temperature (22°C ± 2°C) and humidity (50% ± 5%) conditions with available food and water *ad libitum*. All the associated experiments were approved by the Research and Ethics Committee of Isfahan University of Medical Sciences in compliance with the international guiding principles for biomedical research involving animals in 1996 (NIH Publications No. 80–23, 1996 Rev).

Rats were randomly allocated to the following three groups (n = 6 in each group): Control (Co), social stress (SS), and isolation stress (IS) groups. Then, they were subjected to 7 days of subchronic social and isolation stresses.

Stress paradigm

To induce social stress, rats were transferred to the new cage with new neighbors for every 24 h as psychological stress.[24] To induce isolation stress, rats were kept in individual cages without any other neighbors.[24,25] Similarly, to induce social and isolation stresses, the rats were subjected to 7 days of subchronic social and isolation stresses.[16,18,26]

Food intake paradigm

The simplest paradigm for investigating the food intake is to record the mass of food eaten during the fixed period.[27] The stress lasted for 7 continuous days. For feeding measurements, the rats fasted for 16–18 h on day 7 of the experiment. At the end of experiments, on day 8, the rats were transported to the laboratory at least 1 h before the beginning of the feeding trial. The weight of food pellets was measured on an hourly basis and for a 3 h.[28,29] Subsequently, the rat was individually placed in a transparent Plexiglas cage with a thick white paper lining at the bottom and allowed to have access to a premeasured amount of regular laboratory chow. Therefore, over three continuous hours, the rats were removed from the first test cage and placed into their new test cage after each hour.[30,31] The amounts of food left in the first test cage, including crumbs, were measured and the amounts consumed were calculated. Furthermore, the feeding trials were done normally between 9:00 am and 12:00 on rats deprived of food for 16–18 h.

Measurement of body weight differences

Animal body weights were measured on days 1 and 7 of the experiment and the body weight differences (BWD = BW_{Day7}−BW_{Day1}) were evaluated.

Assessment of feeding biomarkers levels

In the current study, the levels of feeding biomarkers containing the serum levels of ghrelin and leptin as well as blood glucose level were measured. Hence, at the end of the experiments, day 8, rats were euthanized by light anesthesia. Tail blood sampling technique was used to collect blood (at the amount of 500 μl) from the rats at 8:00 to 9:00 am. On the fasting day, the blood glucose levels were measured using a glucometer (On Call Plus Co., USA). In addition, blood samples for hormonal analysis were collected in plastic vials and centrifuged at 6000 rpm for 20 min. Serums were separated from blood samples and stored at −80°C until hormones (the ghrelin and leptin) analysis. The commercial enzyme-linked immunosorbent assay kit (Zellbio Co., Germany) was used to assess the serum ghrelin and leptin levels.

Statistical analysis

The feeding study trials and other variables (e.g., levels of ghrelin, leptin, glucose and BWD) of the various groups (i.e., between-group comparisons) were compared using independent t-test. Furthermore, the repeated-measure ANOVA followed by least significant difference (LSD) *post hoc* test was used for food intake trend between experimental groups. The 3-h consecutive food consumption for comparing the food intake in 2 h (food intake of 1 vs. 2 h, 2 vs. 3 h, and 1 vs. 3 h) within the groups were analyzed using the paired Student's t-tests. Results are presented as mean ± standard error of the mean. The $P < 0.05$ was considered as statistically significant. Ultimately, the calculations were performed using SPSS 21 (SPSS Inc., Chicago, IL, USA).

Results

Effects of subchronic stresses on food intake of three continuous hours

Statistical analysis on the food intake of three continuous hours revealed that the food intake significantly ($P < 0.05$) reduced at the 1st h of measurement in the subchronic SS group compared to the Co group. Furthermore, the

consumption of food intake in the subchronic IS group significantly ($P < 0.001$ and $P < 0.01$; respectively) decreased in the 2nd h compared to the Co and SS groups. In addition, the food intake changes at the 3rd h showed no significant reduction in both SS and IS groups [Figure 1].

Effects of subchronic stresses on cumulative food intake

The results show a decline of cumulative food intake in both IS and SS groups. Moreover, the cumulative food intake significantly ($P < 0.05$) showed a reduction only in the IS group compared to the Co group [Figure 2].

Effects of subchronic stresses on food intake trend

Based on the repeated mature ANOVA and *post hoc* LSD's results, there was a statistically significant ($P < 0.05$) difference in the IS group when compared with the Co group [Figure 3].

Food intake of all three trials (i.e., 1 vs. 2 h, 2 vs. 3 h, and 1 vs. 3 h) was analyzed by the paired Student's *t*-tests [Figure 3]. The analyses revealed statistically significant differences in food intake 1 vs. 2 h, 2 vs. 3 h, and 1 vs. 3 h in the experimental groups [Figure 3].

The food intake of paired trials 1 vs. 2 h in the Co, SS, and IS groups showed significant ($P < 0.01$, $P < 0.05$, and $P < 0.001$, respectively) decreases [Figure 3]. In these groups, comparison of food intake of 2 h vs. 3 h showed statistically significant ($P < 0.05$) decreases only in the IS group [Figure 3]. Furthermore, in the Co, SS, and IS groups, the food intake of 1 h vs. 3 h showed significant ($P < 0.01$, $P < 0.01$, and $P < 0.001$, respectively) decreases [Figure 3].

Measurement of body weight differences

A declined food intake trend and body weight drop appeared in both SS and IS groups [Figure 4]. Although a slight body weight was gained in the Co group, the body weights were decreased in both subchronic IS and SS groups.

The results indicated no statistically significant difference between the SS and Co groups with regard to the BWD [Figure 4].

As shown in Figure 4, the BWD was significantly ($P < 0.05$) lower in the IS group compared to the Co group.

Assessment of feeding biomarkers levels

The serum ghrelin level significantly ($P < 0.05$) decreased in the IS group compared to the Co group. Furthermore, a slight or no significant reduction was observed in the SS group [Figure 5a].

The serum leptin level did not show a statistically significant increase ($P > 0.05$) in the IS group while in the SS group, the serum leptin level was similar to the Co group [Figure 5b].

As can be noted, the blood glucose had no significant increases in both the SS and IS groups compared to the Co group, suggesting partial hyperglycemia in both SS and IS groups [Figure 5c].

Discussion

The effect of subchronic psychological stress was not clear on the food intake and feeding biomarkers such as levels of leptin, ghrelin, and glucose. Hence, the present study evaluated whether two types of subchronic (social and isolation) stresses could be mediated through the changes of serum levels ghrelin and leptin as well as blood glucose level on the energy homeostasis (food intake) and body weight.

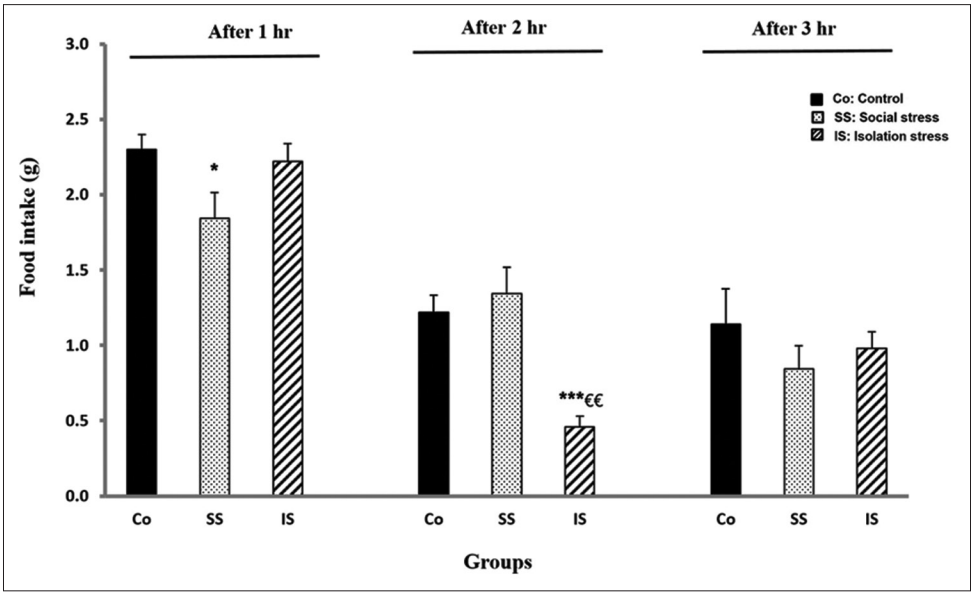

Figure 1: Comparison of the food intake (g) in the experimental groups after 1, 2, and 3 h. Results are expressed as mean ± standard error of mean (independent samples *t*-test). **P* < 0.05 and ****P* < 0.001 compared to control group, €€*P* < 0.01 compared to social stress group

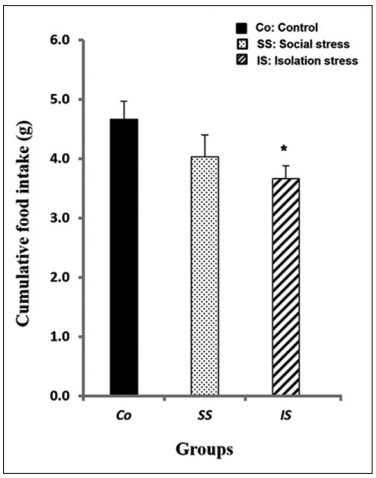

Figure 2: Comparison of the cumulative food intake (g) in the experimental groups. Results are expressed as mean ± standard error of mean (independent samples *t*-test). **P* < 0.05 compared to control group

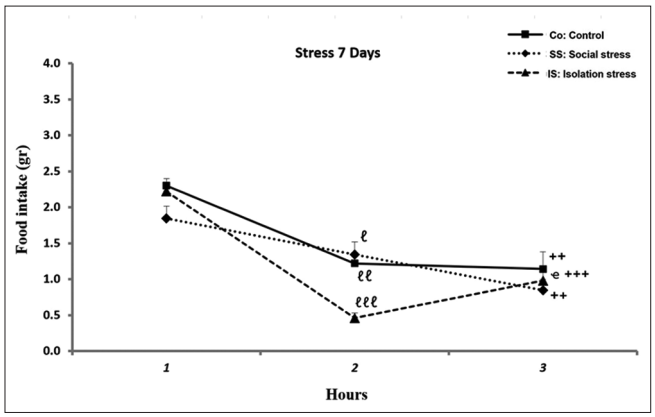

Figure 3: Comparison of the 3-h food intake trend (three continuous hours) in the experimental groups; results are expressed as a mean ± standard error of the mean (repeated measure one-way ANOVA followed by least significant difference's *post hoc* test and paired Student's *t*-test for comparing of the food intake in comparison of 2 h in each group). $^{\ell}P < 0.05$, $^{\ell\ell}P < 0.01$, and $^{\ell\ell\ell}P < 0.001$ food intake value in 1 versus 2 h; $^{e}P < 0.05$ food intake value in 2 versus 3 h; $^{++}P < 0.01$ and $^{+++}P < 0.001$ food intake value in 1 versus 3 h

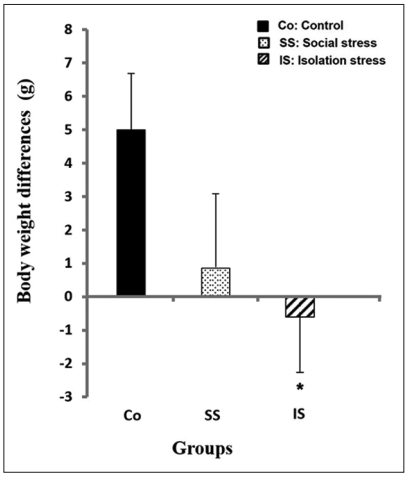

Figure 4: Comparison of the body weight differences (g) in the experimental groups. Results are expressed as mean ± standard error of mean (independent samples *t*-test). **P* < 0.05 compared to control group

According to the results of the current study, the amount of food intake decreased in subchronic social and particularly isolation stress groups [Figures 1 and 2], as a declining food intake trend was observed in both these groups [Figure 3]. Furthermore, BWD followed the feeding behavior in the subchronic stress conditions [Figure 4]. It is possible that the body weight loss resulted in the reduction in food intake and enhancement probably of body metabolism in subchronic stress conditions. Some animals and humans studies demonstrated that the food intake either increases or decreases in response to the different kinds of stress.[32-35] Previous studies have identified various aspects of feeding behaviors including the increase in food intake in repeated restraint stress, the body weight loss in an inescapable shock condition, and anorexia in immobilization stress.[36-38] According to Ranjbar *et al*., stress is divided into different subsets based on the duration of acute stress, subchronic stress (mid stress), and chronic stress. They demonstrated that subchronic stress (7 days) has the most deleterious emotional stress.[12,16,17] In this regard, previous studies also have shown that acute stress is associated with the reduction of food intake,[4,39] while chronic stress increases the food consumption.[40] Moreover, it seems that changes of food intake are related to the stressor's characteristics such as type stress, duration of stress, the intensity of stress, and the individual's stress characteristics, metabolic state, and dietary in stress condition.[4,6,14] Furthermore, Ranjbar *et al*. reported the effect of three durations (1, 7, and 21 days) of restraint stress including acute, subchronic, and chronic stresses on the alternations of BWD. Moreover, they proposed that there were more changes in subchronic restraint stress on BWD.[17] Collectively, the findings of the current and previous studies suggested that stress duration (even with different kinds of stress) is the main factor that affects the BWD. In addition, it is proposed that the subchronic stress corresponds to the alternation of food intake responses similar to acute stress and reduces food intake and body weight.

Another finding of this study is that the serum ghrelin level significantly decreased in isolation stress groups, although this reduction was not statistically significant in the subchronic social stress [Figure 5a]. Consistent with these data, Currie *et al*. reported some interactions between ghrelin and corticotropin-releasing (CRH) hormone for controlling of the neural circuits of stress and feeding behaviors.[41] Since the ghrelin plays an important role in adjusting hypothalamic–pituitary–adrenal (HPA) axis, the potential role was considered for ghrelin as a stress feedback signal.[42] In this connection, Saegusa *et al*. reported that the serum ghrelin level decreased after 7 days of novelty stress.[43] They suggested the increases in CRH resulted in a declined ghrelin level, leading to the sustained food intake reduction.[43] However, other researches indicated an opposite finding between the ghrelin level and food intake.[44-46] Some previous studies demonstrated that

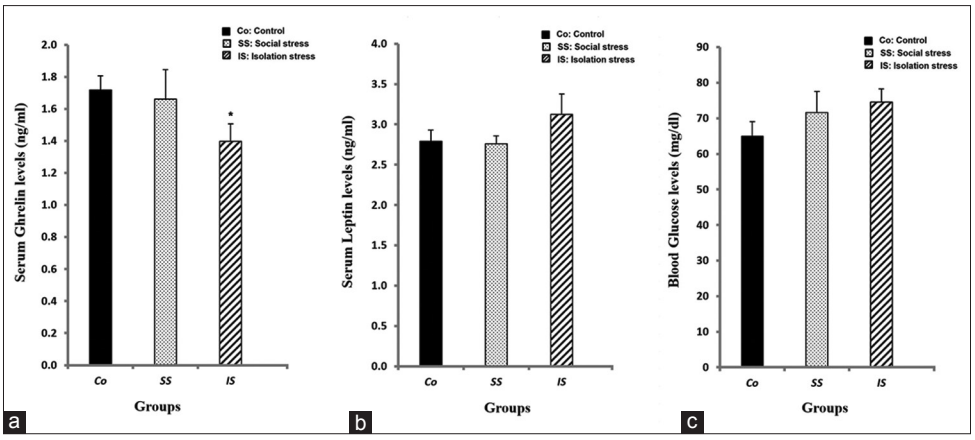

Figure 5: Comparison of (a) the serum ghrelin level (ng/ml), (b) the serum leptin level (ng/ml), and (c) the blood glucose levels (mg/ml) in the experimental groups. Results are expressed as the mean ± standard error of the mean (independent samples *t*-test). **P* < 0.05 compared to control group

the elevated serum ghrelin level helps to an individual for adaptation to chronic stress but at the expense of increased eating food.[45] Therefore, it is possible that subchronic isolation stress suppresses appetite and food consumption by the reduction of serum ghrelin level in the current study.

According to other data presented in this work, the serum leptin level showed no significant increase in subchronic social and isolation stress groups [Figure 5b]. In contrast, Ortolani *et al*. showed that the anorexigenic effect of footshock stress is irrelevant to leptin serum elevation.[35] Meanwhile, Bernier *et al*. reported that leptin regulates the food intake in hypoxic stress.[47] Furthermore, another study reported that leptin decreased the food consumption by inhibition of orexigenic signals like NPY and agouti-related peptide as well as the expression of the anorexigenic signals such as proopiomelanocortin.[48,49] Nevertheless, according to a previous study, the changes in the leptin level (as a regulatory link between energy homeostasis and the HPA function) depend on various factors such as different psychological stressors.[50,51]

We found a nonsignificant hyperglycemia in both subchronic stress groups [Figure 5c]. In contrast, some studies demonstrated sympathetic and/or glucocorticoids activation can increase the blood glucose under acute stress conditions.[39,52] Furthermore, Shiiya *et al*. reported that the elevated blood glucose level can decrease the serum ghrelin level.[53] Moreover, in the present study, the changes in leptin and glucose levels were in line with other studies, but statistically nonsignificant. Therefore, based on the results of the present study, it is logical to expect that the slightly and nonsignificantly elevated serum leptin and blood glucose levels may have a contributory role on the decrease in ghrelin level for anorectic effects in the subchronic stress conditions. These differences depend on the ability to adapt to stress; an ability that is related to sex, age, genetic makeup, stress duration, and environmental influences.[54]

Conclusions

Subchronic isolation stress seems to be more destructive with respect to subchronic social stress on food intake reduction. In addition, it is possible that the reduced food intake and body weight loss result in the decreased serum ghrelin level, but not the elevated serum leptin and blood glucose levels in subchronic stress conditions. Nevertheless, these factors may help decrease the ghrelin level for anorectic effects in the subchronic stress conditions. Accordingly, further studies need to be carried out to clarify the neuronal pathways by which psychological stresses exert either inhibitory or stimulatory effects on food intake as a function of time.

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Conflicts of interest

There are no conflicts of interest.

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