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A N I N T E R N A T I O N A L J O U R N A L

Effect of Intermittent Feeding on Gonadal Function in Male And Female NMRI Mice During Chronic Stress

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ABSTRACT

Stress can inhibit gonadal activity via Hypothalamus-Pituitary-Gonad (HPG) axis activity suppression. In the present study, effects of intermittent feeding (IF) on gonadal function under stress in male and female mice were evaluated. Twenty eight male and twenty eight female mice's were divided into four groups. The control group received adequate food and water without stress. The second group received four days of electric shock without food deprivation. The third group was deprived of food two hours/day for a week, and the fourth group was deprived of food (2 hours/day for seven consecutive days) and then electric foot shock stress was applied to them for four days. Blood samples were collected from all animals for plasma testosterone, estrogen and/or Interlukin-6 (IL-6) evaluation. The animals' gonads were also removed and fixed for the measure of their weight. Results showed that stress reduces both testosterone and estrogen levels, whereas IF did not change the hormone levels. In addition, stress increases blood IL-6 concentration. The combination of IF and stress, increased the hormone levels in animals. Stress and IF alone had no significant effect on gonadal weight in the male mice, whereas stress decreased gonadal weight in the females. Combination of stress with IF increased gonadal weight in both male and female mice. In conclusion stress showed a negative effect on gonadal function in both animals with more effect on females. Intermittent feeding inhibits the stress effect and even promotes the gonadal function in both sexes. The effect may be due to IL-6 reduction.

Key Words: Estrogen; Gonad; IL-6; Intermittent Feeding; Stress; Testosterone

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INTRODUCTION

Stress, especially chronic stress, has wide ranging health outcomes. Stress has also been identified as a predisposing and exacerbating factor in many medical conditions such as psychological disorders, immune system dysfunction, cardiovascular diseases and metabolic complications [1-3]. Stress factors stimulate Hypothalamus-Pituitary-Adrenal gland axis (HPA) through Corticotropin-Releasing Hormone (CRH) in hypothalamic Para-Ventricular Nucleus (PVN); this neuropeptide stimulates secretion of Adrenocorticotropic Hormone (ACTH) from the anterior pituitary, leading to the release of glucocorticoids from the adrenal gland [4, 5]. Steroid hormones can pass through the blood-brain barrier, and affect processes such as neurogenesis, synaptogenesis, dendritic development, and apoptosis [6].

Studies have indicated the presence of CRH and its receptors in the reproductive system [7]. Also, glucocorticoids released from adrenal glands, due to stress, inhibit the production of testosterone in leydig cells [8]. HPA axis has been found to inhibit the activity of the hypothalamus-pituitary-gonad (HPG) [9-11]. Consequently, reproductive activity and sexual desire decrease in both sexes due to chronic stress [10]. Studies have also demonstrated that gonadal hormones can directly affect the function of the HPA axis. For instance, testosterone suppresses HPA axis [8]. On the contrary, estrogen reinforces this axis in many different ways [7]. The activity of gonadal-steroidal hormones, especially testosterone, modulates HPA activity, and thus prevents the harmful effects of HPA activity on the reproductive system function [12].

Intermittent feeding (IF) (reduced calorie intake) has been proposed as a longevity-increasing factor by making regular circadian cycles and having a positive effect on the brain's suprachiasmatic nucleus function that controls circadian rhythms [13-14]. Studies on rats have shown that reduced calorie intake regulated circadian rhythm better compared to the rats that were fed normally, and increased their longevity [15]. Restricted calorie intake (without causing malnutrition) can protect the brain against neurological damages and disorders, and protects neurons against degeneration, stimulation of neurogenesis, increased synaptic deformation, and increased brain capability for resuming normal function following damage [16-18]. Also, restricted calorie intake maintains immune system in the best condition, and reduces the progress of autoimmune diseases such as lupus erythematosus and other autoimmune diseases in both humans and animals [19-21]. This nutritional restriction can cause recovery from encephalomyelitis [22, 23]. Finally, restricted calorie intake promotes brain health and reduces complications due to diseases such as Alzheimer's disease, Parkinson diseases, Huntington disease [24], as well as complications due to stroke in animal and human models [15-18]. The proper function of the reproductive system is an important indicator of health in every community, and improving function of this system is one of the objectives of various health sectors in different countries [9]. On the other hand, proper functioning of the reproductive system depends on limiting the amount of stress. Accordingly, and given that in previous studies, intermittent feeding had led to inhibition of metabolic effects of stress in male and female mice [25], the present study aims to investigate the effect of intermittent feeding on the destructive effects of stress on the reproductive system in male and female mice. In this regard this study tries to show a possible role for IL-6 as an indicator for inflammation.

MATERIAL AND METHODS

Animals

In this study, male and female NMRI mice weighing 27±3 g, procured from Tehran's Pasteur Institute and housed in cages (8 mice in each group) with food and water ad lib and 12-hour light/dark cycle. Mice were transferred to laboratory to adapt to new environment one hour before the start of the tests. All procedures were approved by the Animal Care and Use Committee, Baqiyatallah University of Medical Sciences, Tehran, Iran.

Groups

Animals were tested in male and female groups. Prior to the experiments, animals were randomly divided into four groups (n=8 for each group). Group one received food and water *ad lib* for the first seven days, and then housed for one hour per day for four days in a switched off stress-inducing device (Communication box, made by Borje-Sanat Company-Iran). Group two was treated the same as group one, except that stress was induced (voltage 40mv, frequency 10Hz, for 100 seconds). Group three was deprived of food for two hours per day for seven days (Ghanbari *et. al.*, 2015). Group four was treated the same as group three, but also received stress for four days.

Stress Procedure

The stress device (communication box) comprises nine separate compartments (50×16×16cm for length, width, height) and is made of Plexiglas, with transparent walls containing small holes to maintain visual, olfactory, and auditory contact, with 4mm diameter steel bars, spaced 1.3cm from each other on the floor. Electric shock (10 mV voltage, 10 Hz frequency and 60 s long) is transmitted to the sole of the feet of the animals through these bars. Intensity and duration of the shock are controlled by a computer connected to the device. To adapt to the new environment, animals were transferred to the laboratory one hour before tests, and remained inside the device 30 minutes before and 30 minutes after inducing stress. The animals were randomly divided into control and stress groups. The animals in the control group were also placed in a switched off communication box for 30 min. Stress induction continued for four consecutive days.

Blood Sampling

On the last day, blood samples were taken from the animals' retro-orbital sinus at the corner of the eye, and centrifuged at 3000 rpm and 4 °C for five minutes. Blood plasma was collected and frozen at -80 °C for testosterone, estrogen and IL-6 assessment. Next, the effects of stress and intermittent feeding on these hormones were assessed using testosterone, estrogen and or IL-6 (Mouse IL-6 ELISA Kit; ab100712) measuring kit and ELISA reader. After completion of tests, animals were anesthetized with a high dose of ketamine, and their gonads were removed and fixed in 4% formalin solution. After 30 days, gonads were removed from formalin and their weights were measured.

Data Analysis

The data was analyzed using the SPSS version 19.0 software. Data were tested for significance using a one-way ANOVA, followed by a Tukey post hoc test. The data from these experiments were reported as mean ± standard errors of mean (SEM) for each group. $P < 0.05$ was considered a statistically significant difference.

RESULTS

Effect of intermittent feeding, with or without inducing stress, on the concentration of plasma testosterone and estrogen

Results showed reduction in testosterone and estrogen in both sexes due to induced chronic stress. On the other hand, intermittent feeding had no effect on these hormones, but chronic stress coupled with intermittent feeding increased levels of these hormones, and this was statistically significant ($P < 0.05$) in both sexes (fig. 1a and 1b).

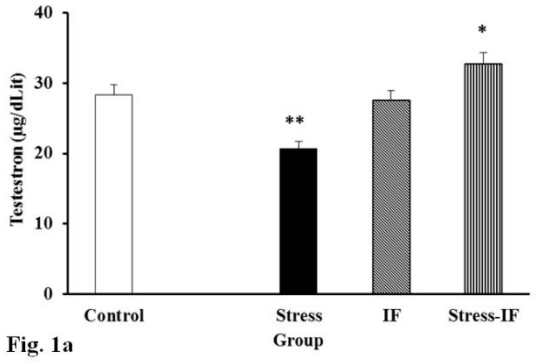

Fig. 1a

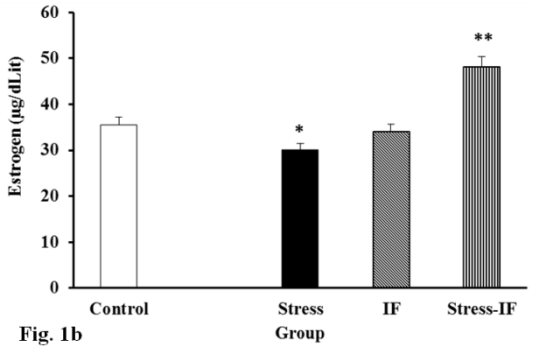

Fig. 1b

Figure 1 - a: Plasma testosterone changes in the different groups of the male animals. It is noted in the figure that stress reduces whereas intermittent feeding increases plasma testosterone levels. Each point represents the mean±SEM for seven animals. *P<0.05 and **P<0.01 difference from the control group. **b:** Plasma estrogen levels in the female mice in different groups. It is clear that stress reduces whereas intermittent feeding increases plasma estrogen concentration. Each point represents the mean±SEM for seven animals. *P<0.05 and **P<0.01 difference from the control group.

Effect of intermittent feeding with or without induced stress on weight of gonads in male and female mice

In this part, results showed a reduction in weight of the gonads in both sexes due to induced chronic stress, which was not statistically significant ($P < 0.05$) in male mice, but highly significant ($P < 0.05$) in female mice than the control group. On the other hand, intermittent feeding alone had no effect weight change in gonads of both sexes. But in stress+IF groups the weight of the gonads increased in both sexes compared to the stress group in which this change was statistically significant ($P < 0.05$) (fig. 2a and 2b).

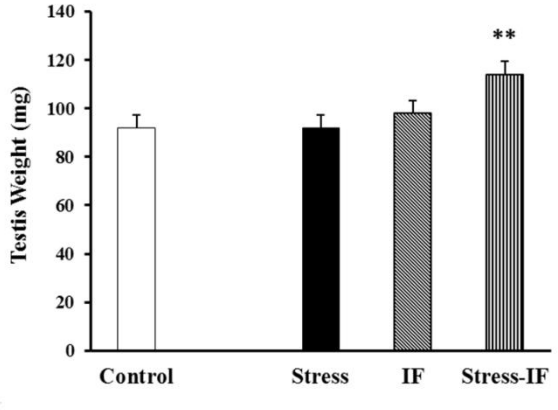

Fig. 2a

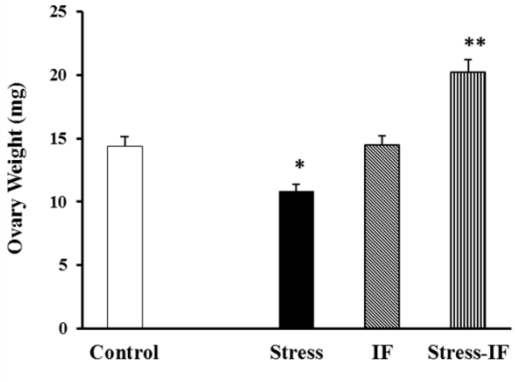

Fig. 2b

Figure 2 - a: The effect of chronic stress on gonadal weight in the male mice. Stress had no effect on gonadal weight in the male mice, but intermittent feeding increased it. Each point represents the mean±SEM for seven animals. **P<0.01 difference from the control group. **b:** Effect of chronic stress and intermittent feeding on gonadal

weight in the female animals. Stress reduces, but intermittent feeding increased gonadal weights in the female mice. Each point represents the mean±SEM for seven animals. *P<0.05 and **P<0.01 difference from the control group.

Effect of intermittent feeding with or without induced stress on plasma IL-6 concentration

Results indicated an elevation in plasma IL-6 in male mice due to induced chronic stress, which was statistically significant (P < 0.001) than control group, but intermittent feeding during chronic stress significantly reduced IL-6 in plasma than stress group (fig. 3a). Plasma IL-6 due to induced chronic stress had no significant changes (P < 0.05) in female mice in control, stress, IF and stress+IF groups (fig. 3b). On the other hand, intermittent feeding had no significant effect on IL-6 change in female mice during stress.

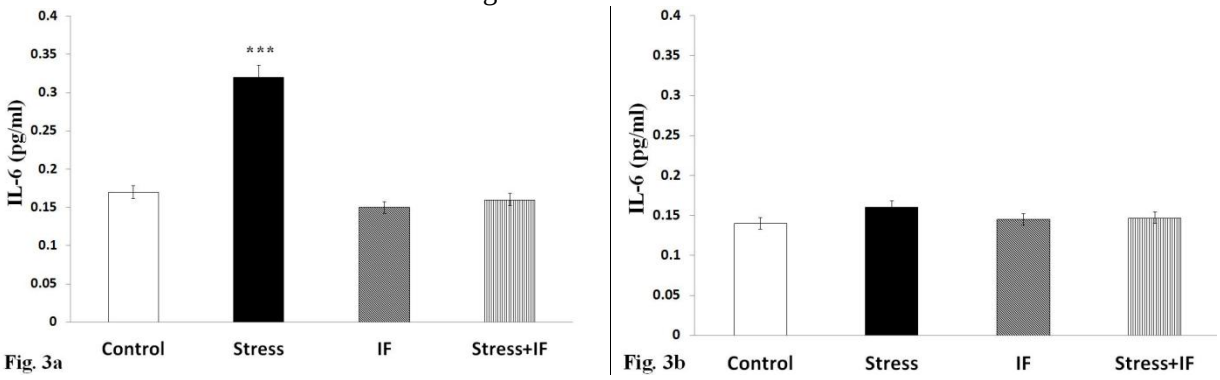

Figure 3 - a: The effect of chronic stress on plasma IL-6 concentration in male mice. Stress increased plasma IL-6 concentration in the male mice but intermittent feeding had no effect. Each point represents the mean±SEM for seven animals. ***P<0.001 difference from control group. **b:** The effect of chronic stress on plasma IL-6 concentration in female mice. Stress slightly increased plasma IL-6 concentration in the female mice but intermittent feeding had no effect. Each point represents the mean±SEM for seven animals. *P<0.05 difference from control group.

DISCUSSION

This study examined the effect of stress and intermittent feeding on the changes in secretion of main sex hormones from the gonads and the weight change in gonads. Moreover, we evaluate the changes in plasma IL-6 as one of the inflammation mediators in this regard. Results shows the adverse effects of stress and positive effects of intermittent feeding on gonad activity and neutralization of stress effects as well as the IL-6 concentration. The present study demonstrates that stress induced through electric shock to the sole of the mice (with physical and psychological components), reduces sex hormones in male mice, and significantly more so in females. Also, this study shows that stress can cause weight loss in gonads in both sexes, with higher efficiency in females. Previous studies have shown that stress system and reproductive system interfere with each three levels of hypothalamus, pituitary, and glands (gonads and adrenal), and activity of one reduces the activity of the other [9, 11]. For instance, hypogonadism has been found very common in people with Cushing's syndrome (relating to severe hypersecretion of glucocorticoid hormones from the adrenal glands) [26, 27]. Moreover, corticosterone inhibits sexual activity in female rats by reducing activity of GnRH-secreting cells in hypothalamus, FHS and LH in pituitary, and estrogens in ovaries and thus may terminate development of endometerium in females. Similar stress effects have been observed in males, as well. For instance, psychological stress due to loss of a loved one has led to reduced sperm production in men [9]. Also, glucocorticoids have been found to

inhibit secretion of testosterone from leydig cells. Stress, can reduce testosterone production and secretion in rats [8]. These studies are consistent with the results of the present study and point to the hypoactivity of gonads in both sexes due to the effects of stress hormones (in all three levels discussed above) in the reproductive system. More importantly, the present study indicates that the female reproductive system is more sensitive to the effects of stress than the male reproductive system because stress causes less gonad weight loss in male mice compared with female mice. Previous studies have shown that female gender is more sensitive to stress than male, and this has been attributed to the much greater number of small CRF-secreting cells in the hypothalamus PVN nucleus in females compared to males. Thus, in females, stress causes greater secretion of CRF, leading to greater secretion of ACTH and corticosteroid hormones in response to stress in females [5]. The present study indicates the potential risk of severe psychological and physical stress on gonads because of the gonad atrophy that occurs following stress and its degeneration by stress hormones can potentially reduce reproductive power. Intermittent feeding was able to reduce the effects of stress in gonad weight loss, but it also caused weight gain, while intermittent feeding alone had no effect on gonad weight. No study has yet been conducted on the effect of intermittent feeding on gonad weight. However, it seems intermittent feeding has no effect on gonad weight, which is a positive point about this method used in the present study. Additionally, after inducing stress in mice with food deprivation, the weight of the gonads did not decrease, but it significantly increased. Currently, the reason for this weight gain is unknown, and it should be investigated in future studies with a cellular and molecular approach at hypothalamus, pituitary and adrenal levels. It is important to know that one of the main reasons for sexual dysfunction in both sexes is the reduction in physical size of the glands and their weight, which may be attributed to numerous factors including stress [9, 11]. Since stress is an inseparable part of life, reducing calorie intake (less feeding) can be recommended as a method for reducing the effects of stress without side-effects. In this study, the weight of the animals did not decrease due to intermittent feeding, and no change was observed in weight (data not shown) in the intermittent feeding-plus-stress group. Thus, gonad weight gain in intermittent feeding-plus-stress group should be further investigated.

In the second part of the study, in line with the first part and also with many previous studies, stress was able to drastically reduce the concentration of plasma sex hormones in both sexes. The reduced levels of hormones may be attributed to stress-induced weight loss or even the effect of stress hormones on the gonads and the inhibition of secretion of sex hormones. According to previous studies, stress-induced release of glucocorticoid hormones from the adrenal glands can reduce the production and secretion of testosterone by affecting sertoli cells in male gonads [28-31]. This effect may be due to inhibition of β11-hydroxysteroid-dihydrogenase in sertoli cells [30]. It may even be due to the effects of stress hormones at the hypothalamus level because previous studies have shown that secreted CRF in hypothalamus during stress can inhibit secretion of GnRH. Since the concentration of HPA axis hormones was not measured, no comment can be made on which of these hormone had more effect.

In female mice, plasma estrogen concentration was significantly higher in the control group compared to the group receiving stress. Previous studies have shown that stress can reduce ovarian activity, induce amenorrhea, reduce libido, reduce fertility and reduce the effect of estrogens in stimulating endometrial development [11]. In the present study, stress was able to reduce plasma estrogen concentration, which may also be due to stress-induced gonad weight loss. Interestingly, intermittent feeding alone had no effect on the concentration of hormones in male and female mice, and this can be considered a positive point of the method used in the present study. On

the other hand, intermittent feeding coupled with stress inhibited the effect of stress, and also increased plasma concentration of both hormones in both sexes. Despite the gonadal weight gain due to intermittent feeding and stress, the gonads appear to be functional and even show improved function in secreting sex hormones. The results obtained in this part were more significant in females compared to males, indicating greater sensitivity of the female mice. Kumar and Gurcharan (2013) showed that sex-based difference in animals responding to intermittent fasting dietary restriction (IF-DR) regimen as the reproductive changes were more pronounced in IF-DR females as compared to their male counterparts [32].

In the last section of these results, data indicated that plasma IL-6 is higher in the stressed groups than the other experimental and control groups. Stress has been shown to activate the inflammatory cascade, that IL-6 as circulating biomarkers of inflammation plays a role in both acute and chronic stress responses, which stress can induce profound elevates in circulating IL-6 [33, 34]. Some studies showed that nerve injury increased the inflammation factor in the central nervous system [35-39]. The role of inflammatory mediators in gonadal abnormal function investigated in studies [9-11], which in line with our results. However, new finding is that intermittent feeding can inhibit the stress effect on Il-6 as an inflammatory mediator and possibly reduce its adverse effect on gonadal activity.

CONCLUSIONS

The present study showed that chronic stress has dramatic functional and physical effects on gonads in both sexes, and intermittent feeding (as described above) is capable of inhibiting these destructive effects, and can even improve them. Moreover, these findings indicated that at least, one of the mechanisms which intermittent feeding reduced the effects of stress may be due to inhibition of inflammation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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