The Comparison of the Effects of Acute Swimming Stress on Plasma Corticosterone and Leptin Concentration in Male and Female Rats
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Received: 29 Oct. 2009; Received in revised form: 10 Apr. 2010; Accepted: 2 Jun. 2010

Abstract- The aim of this study was to investigate the effect of acute swimming stress on plasma corticosterone and leptin levels in female and male rats. Thirty-seven adult male (n=20) and female (n=20) Sprague Dawley rats (200-250 g weight) were used. The leptin and corticosterone levels were measured following swimming stress (10 minutes) or no stress. Plasma leptin and corticosterone were measured by ELISA system. The plasma leptin and corticosterone levels were significantly increased in female and male rats by swimming stress. Plasma leptin level was not correlated significantly with plasma corticosterone in all groups. There were no sex differences in leptin level among stressed and non-stressed rats. The results suggest that changes in plasma leptin level could not be associated with stimulation of corticosterone secretion from adrenal glands and leptin secretion is not sex dependent.

Keywords: Stress; Leptin; Corticosterone; Rat

Introduction
In response to stressful events hypothalamus is activated to secrete corticotrophin releasing factor (CRF) from paraventricular nucleus. CRF can causing the secretion of adrenocorticotrophin (ACTH) from anterior pituitary, in turn, stimulates the release of glucocorticoids from adrenal glands. Activation of HPA axis is responsible for maintenance of homeostasis during stress. Recent investigation demonstrated existence of a classical endocrine loop between HPA axis and adipose tissue. Glucocorticoids can stimulate leptin secretion from adipocyte, where increase in leptin level may exert inhibitory effect upon the HPA axis (1-3). In spite of numerous studies in related to effect of stress on leptin level, most of them have been done in males. It is known females respond differently to stress (4-6) and HPA activity is gender specific (5). According to the above considerations, the aim of this study was to investigate effect of acute stress on leptin level in both sexes (female and male rats). In another study has been shown inverse relationship between corticosterone and leptin in unstressed animal models (7). Therefore, we sought to answer whether there is or not such relationship between two variables in stressed rats.

Materials and Methods

Animal
Thirty seven adult (20 male and 20 female) Sprague-Dawley rats (200-250 g weight) were used. The experimentally animals were housed in groups of 3-4 in home cages made of plexiglass material with the floor covered with sawdust. They were maintained under standard condition (temperature 20 ± 2 °C, lights on from 06.00-18.00 hours) with free access to food and water. The animals were randomly divided into four groups (n=10 for each group): group 1, unstressed male rats; group 2, stressed male rats; group 3, unstressed female rats; group 4, stressed female rats.

For inducing swimming stress rats had placed individually in water containing style sink (30 cm height, 60 cm length, 30 cm wide) for 10 min. The temperature of water was ± 25 °C. At the end of the swimming period rats were gently dried before blood collecting. On the day of experiment, trunk blood was collected between the hours of 10:00 and 12:00 a.m. and plasma samples were obtained by centrifugation of blood at 3500 for five minutes (twice). Plasma was frozen until the day of the analysis. Measurement of plasma leptin and corticosterone were done with a commercial rat leptin ELISA (Biovender Inc. Czech-
Slovakia) and rat corticosterone ELISA kit (Boldon, Tyne & Wear, NE35 9PD, UK).

Statistical analysis
All experiments were conducted in accordance with the guidelines Jahrom University Ethics Committee on the use of laboratory animals.

Data were expressed as Means ± S.E.M. To analyze leptin or corticosterone level after stress, student's t-test was used. The correlation between plasma leptin and corticosterone level examined by simple bivariate analysis. Statistical analyses were performed using spss12 software. Threshold of significance was defined at $P<0.05$.

Results
The effect of acute swimming stress on plasma leptin was shown in fig 1. Stress increased circulation leptin concentration in male and female rats ($P<0.001$). In addition, stress increased significantly corticosterone concentration in male ($P<0.05$) and female ($P<0.01$) rats (Figure 2).

There was no significant difference in leptin level between non-stressed female and male rats. In addition, there was no significant difference in leptin level between stressed female and male rats. Plasma leptin level was not correlated significantly with plasma corticosterone in all groups.

![Figure 1](image1.png)  
**Figure 1.** The effect of acute swimming stress on plasma leptin concentration in male and female rats. (Data are represented as Mean ± S.E.M) *** $P<0.001$

![Figure 2](image2.png)  
**Figure 2.** The effect of acute swimming stress on plasma corticosterone concentration in rats. (Data are represented as Mean ± S.E.M) *$P<0.05$, **$P<0.01$
Discussion

The present results show that acute swimming stress increases level of leptin and corticosterone in female and male rats. Increase in corticosterone level confirmed stress exposure. The data from this study are in consistent with similar other researches (8, 1, 9). Stress activate sympathetic system and hypothalamo-hypophyseal-adrenal axis (10, 11). There is evidence which suggests, stimulation of sympathetic system inhibits leptin secretion from adipocyte tissue (12) and glucocorticoids (13, 14) and possibly ACTH (14) stimulates leptin biosynthesis and secretion. In our study, we did not observe any correlation between plasma concentration of leptin and corticosterone. It is consistent with some previous reports (15, 16). Ceballos and colleagues reported that correlation between leptin and corticosterone were strain specific (17), but it is reasonable to speculate that the swimming stress in rats resulted in enhanced leptin levels as stress related hormone. Several studies support this speculation (16, 18, 19).

There is evidence for gender difference in the HPA axis responses to stress. In response to alcohol intact female rats secret significantly more ACTH and corticosterone than intact males (20). Also Rivier observed in response to shock intact females secret significantly less ACTH than intact females (5). In present study, stressed female rats displayed higher corticosterone secretion levels than stressed male rats. But this difference was not significant between two groups.

It seems gender difference in HPA axis response were not always present and it dependsents to kind of stress (21, 22) or even the stress intensities (23). Male rats release more ACTH than female rats in response to alcohol (3g/kg) (5) but after the injection of smaller doses of alcohol (0.2-1.8 g/kg) female rats release more ACTH and corticosterone in response to alcohol (20). In another study have been shown, psychosocial stress induced significant increase in salivary free cortisol with no significant differences between men and women (22).

To date, a few studies assess whether gender-associated differences exit in plasma leptin level (24, 25, 17). These researches have shown leptin level in female rats is more (17) or less (24, 25) than male rats. But our data did not show sex differences in plasma leptin level between male and female rats. In this regard, many more studies will be necessary to confirm whether leptin secretion by adipose tissue is gender-dependent.

References


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