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Reproductive Hormonal Changes after Incremental Exercise in Female Rats

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Abstract: Regular physical exercises can affect hormonal system of female athletes. This study evaluated the reproductive hormonal changes after an incremental exercise in female rats. Forty female 2 months old Sprague Dawley rats (200±5 g) were randomly divided into 4 equal groups including group 1 as control, group 2 undergoing incremental exercise, group 3 receiving supplement soy milk and group 4 undergoing incremental exercise together with a supplement of soy milk. The incremental exercise consisted of running on a flatted treadmill 3 days/weak for 10 weeks. The speed of treadmill gradually increased from 18 to 36 m min^{-1} and the duration of each exercise started from 5 min in the first week and reached 8 min in the last week of exercise. The supplemented soy milk was administered for 10 weeks. After 10 weeks, animals were bled and the level of estrogen, progesterone, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) were determined. The LH level was statistically significant different between groups 1 and 2, 1 and 4, 2 and 3 ($p<0.05$) and groups 3 and 4 ($p<0.05$). FSH level was statistically significant between groups 1 and 4 ($p<0.05$), 3 and 4 ($p<0.05$). Regarding the estrogen level, the difference was not significant between the groups. Progesterone level was statistically significant between groups 1 and 4, 2 and 3, 1 and 3 ($p<0.05$) and 2 and 4 ($p<0.05$). It may be said that changes in the sex hormone are affected by various factors, but what should be considered is the interaction of these hormones. Internal hormonal changes are dependent on the sport history, intensity, duration, level and duration of soy milk supplementation.

Key words: Reproductive hormones, exercise, soy milk, estrogen, progesterone, rat

INTRODUCTION

The pituitary gland is the most important neuroendocrine modulator which regulates many peripheral glands and tissues via the secretion of Anterior Pituitary (AP) hormones including prolactin (PRL), Growth Hormone (GH), Thyrotropin (TSH), adrenocorticotropin and the gonadotropins [Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH)] (Villalobos *et al*., 1997; Karaca *et al*., 2010). Gonadotropic cells are about 10% of the anterior pituitary cells (Yeung *et al*., 2006). They are secreting LH and FSH (Yeung *et al*., 2006). Estrogen action is controlled by gonadotrophin secretion in levels of hypothalamus and pituitary (Shaw *et al*., 2010; Kalantaridou *et al*., 2004).

The female reproductive organ (ovary) is exceedingly sensitive to physiological stress and reproductive abnormalities occur in higher level in women engaged in athletic activity. The prevalence of observed irregularities varies with athletic discipline and level of competition (Kalantaridou *et al*., 2004, 2010; Zangeneh, 2009). Progesterone and estrogen, respectively inhibit secretion of LH and FSH and obstructs the growth of new follicles in ovaries (Ohtani *et al*., 2001; Park *et al*., 2002).

Regular physical exercises that women perform may have hormonal changes leading to menstrual disorders (Warren and Chua, 2008). Some athletes with previous normal menstrual cycle have reported irregularity in menstruation for months or even years after participation

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in exercises such as ballet dancing (69 %) and running (65%) (Warren and Chua, 2008).

In a study by Latour *et al.* (2001), ovariectomy rats showed that exercise with soy milk supplement could increase plasma levels of estrogen and progesterone. Tromp *et al.* (2006) studied the effect of estrogen supplementation and weight bearing exercise on strength of proximal femur bone and showed that after 19 weeks of training program, in ovariectomized rats, the estrogen supplement increased the bone density. Soy milk supplementation with the training program could change the level of sex hormones (estrogen, progesterone, FSH and LH). Therefore, this study aimed to determine the reproductive hormonal changes after an incremental exercise in female rats.

MATERIALS AND METHODS

Animals: Forty female 2 months old Sprague Dawley rats with the weight of 202.33±51.2 g provided from Laboratory Animal Center of Shiraz University of Medical Sciences (Since January 2011 till December 2011) were randomly divided into 4 equal groups including group 1 as control, group 2 undergoing incremental exercise, group 3 receiving supplement soy milk and group 4 undergoing incremental exercise together with a supplement of soy milk.

All animals were housed identically as five rats per cage in a condition of 12 h light/dark cycle with environmental temperature of 21±2°C and relative humidity of 50%. They were fed with standard pellets and had access to food and water *ad libitum*. The enrolled rats were in all menstrual cycles (proestrous, estrous, diestrous, metestrous).

Animal selection: All experiments, subsequent care and sacrifice procedure were all adhered to the same guide lines under supervision of Animal Care Committee of Iran Veterinary Organization. All experiments were carried out under aseptic conditions in Comparative Medicine Research Center of Shiraz University of Medical Sciences. The protocol of anesthesia, surgical procedure, postoperative care and sacrifice were identical for all animals.

Animal groups: The incremental exercise in group 2 and 4 consisted of running on a flatted treadmill (Shiraz, Iran) 3 days/weak for 10 weeks. The speed of treadmill gradually increased from 18 to 36 m min^{-1} and the duration of each exercise started from 5 min in the first week and reached 8 min in the last week of exercise (10th week). All exercises were performed in the morning and for adaptation to further experiments, they underwent exercise for one week (5 times/week) with a speed of 12 m min^{-1} and the duration of 3 min. No electric shock or artificial stimulation was used at during the study.

The supplemented soy milk product (Soyasun-Iran) in the 3rd and 4th groups consisted of a total fat of 2/5 g, cholesterol (10 g), sodium (100 g), total carbohydrate (8.75 g), calcium (100 mg), sugar (5 g) and protein (6.25 g). Supplementation was undertaken with a gavage (1 mL kg^{-1}, 3 times/ week) for 10 weeks. After 10 weeks, all rats were anesthetized with a mixture of 2% xylazine and 10% ketamine (8 and 90 mg kg^{-1}, respectively). They were bled under anesthesia from their heart. The blood sample was centrifuged (Model: Behdad Iran) with 3000 rpm speed for 10 min. The collected serum samples were transferred into tubes and were kept in -20°C for further tests. Then using ELISA test, estrogen (DRG, Germany, 2693), progesterone (DRG, Germany, 1561), LH (Monobind, USA, 625-300) and FSH (Monobind, USA, 425-300) levels were determined.

Statistical analysis: Statistical analysis was performed using SPSS software (version 16.0, Chicago, IL, USA). The normality distribution of data was analyzed by Kolmogorov-Smirnov test and because there was no normality assumption, data was analyzed with Kruskal-Wallis and Mann-Whitney tests and a $p \leq 0.05$ was considered significant.

RESULTS

As shown in Table 1, after 10 weeks, LH level was 0.20±0.29, 0.42±0.26, 0.14±0.04 and 0.56±0.44 MIU mL^{-1} in groups control, exercise, supplement soy milk and incremental exercise together with a supplement of soy milk respectively, while the changes was statistically significant between groups 1 and 2, 1 and 4, 2 and 3 ($p<0.05$) and groups 3 and 4 ($p<0.05$) (Fig. 1a). For FSH level, these were 0.19±0.25, 0.26±0.22, 0.09±0.02 and 0.34±0.27 MIU mL^{-1}, respectively (Fig. 1b). The changes were statistically significant between groups 1 and 4 ($p<0.05$) and 3 and 4 ($p<0.05$) (Fig. 1b).

For estrogen level, the changes were 8.19±10.60, 1.23±1.80, 3.81±5.79 and 2.8±2.89 pg mL^{-1} in groups 1-4, respectively while between groups, the difference was not statistically significant (Fig. 1c). For progesterone level, these were 23.69±9.83, 28.84±11.98, 14.31±10.17 and 14.83±5.12 ng mL^{-1}, respectively and the changes were statistically significant between groups 1 and 4, 2 and 3, 1 and 3 ($p<0.05$) and 2 and 4 ($p<0.05$) (Fig. 1d).

Table 1: The comparison of LH, FSH, estrogen and progesterone after 10 weeks in four study groups (Mean±SD)

	Group			
Variable	1: Control	2: Exercise	3: Supplement	4: Exercise and supplement
LH (MIU mL^{-1})	0.20±0.29	0.42±0.26	0.14±0.04	0.56±0.44
FSH (MIU mL^{-1})	0.19±0.25	0.26±0.22	0.09±0.02	0.34±0.27
Estrogen (pg mL^{-1})	8.19±10.60	1.23±1.80	3.81±5.79	2.80±2.89
Progesterone (ng mL^{-1})	23.69±9.83	28.84±11.98	14.31±10.17	14.83±5.12

Value are as mean±SD

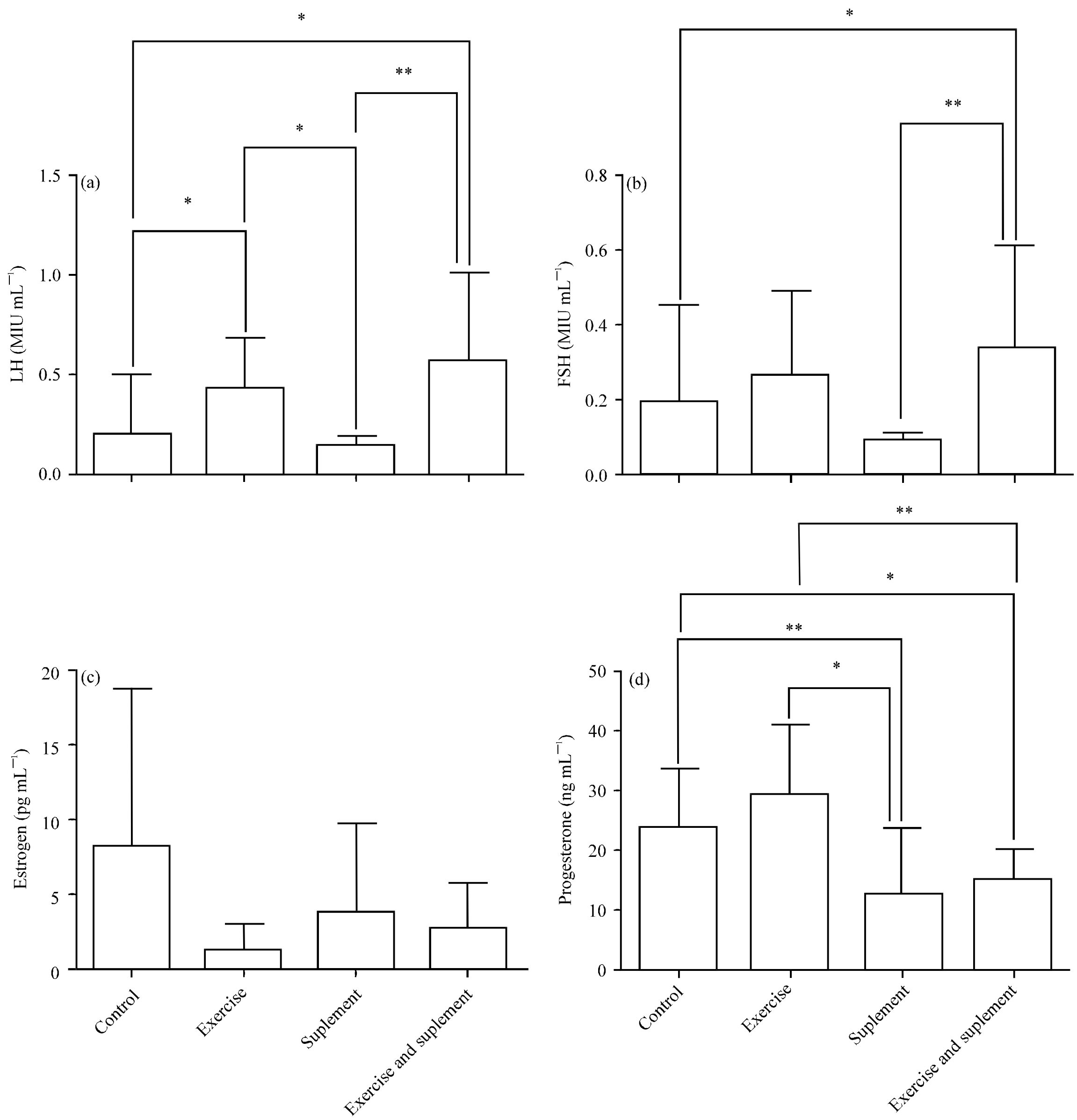

Fig. 1(a-d): The comparison of (a) LH, (b) FSH, (c) Estrogen and (d) Progesterone after 10 weeks in four study groups. *,**Significantly different at p<0.01 and p<0.05, respectively

DISCUSSION

In our study, the lowest plasma estrogen level was noticed in the exercise group (1.23 pg mL^{-1}), whereas the highest level was visible in the control group (8.19 pg mL^{-1}). This issue may be related to the intensity of exercise program, as some researchers have shown that some exercises may lead to a change in estradiol production (Warren and Perlroth, 2001; Kaaks and Lukanova, 2002; Jasienska and Thune, 2001; Keizer *et al*., 1980; Ives *et al*., 2011). In group 4, the estrogen level showed an increase (2.80 pg mL^{-1}) and the secretion of estrogen increased in group 3 in comparisons to group 2 and 4 (3.82 pg mL^{-1}). The difference was not significant between groups 3 and 4 with group 2 due to consumption of soy milk. As soy milk supplementation occurred in group 4, the estrogen level slightly increased in comparison to group 2 (2.80 pg mL^{-1}) and the secretion of estrogen demonstrated a more increase in group 3 in comparison to group 2 and 4 (3.82 pg mL^{-1}, Fig. 1c).

In relation to progesterone level, the highest level was visible in group 2. (28.83 ng mL^{-1}) and the lowest in group 3 (12.39 ng mL^{-1}). Comparison of progesterone and estrogen levels in groups 2, 3 and 4 showed an inverse relationship for these two hormones.

A minimum level of estrogen was seen in group 2 but for progesterone level was the highest in this group (Fig. 1c, d). So progesterone level inversely reduced with consumption of soy milk. Lu *et al*. (2000) showed that the soybean consumption has a relationship with ovarian hormone and Daily consumption of the soya diet reduced circulating levels of 17b-estradiol by 25% and in another study, Lu *et al*. (2001) showed that over the entire menstrual cycle circulating levels of 17b-estradiol significantly reduced by 20% and progesterone by 33% . It seems that physical exercise increased the secretion of progesterone hormone and a significant different was observed between group 2 and 4 regarding progesterone level, so the effect of soy milk supplement in relation to this hormone was more than the effect of exercise and the average level was approximately near the average in group 3 (28.84 ng mL^{-1}).

The highest level of FSH level was visible in group 2 and 3 and the lowest level for estrogen was seen in the same groups too (Fig. 1c, d). Also FSH level in group 3 in comparison to group 2 and 4 was less. Regarding estrogen level in this group, it was more than group 2 and 4, so it could be said that FSH level was affected by the estrogen secretion inhibiting the secretion of FSH too. The highest level of FSH were seen in group 2 and 4 and also for estrogen, the lowest level was noticed in these groups too.

CONCLUSION

Our findings showed that duration, intensity and the amount of the training program with duration of the use of soy milk supplementation could change the sex hormone levels. Our findings showed that exercise may increase the LH, FSH and progesterone levels and decrease the estrogen level. On the other hand, consumption of soy milk supplement resulted into a decrease in sex hormones. We can conclude that changes in the sex hormone are affected by various factors, but what should be considered is the interaction of these hormones. Internal hormonal changes are dependent on the sport history, intensity, duration, level and duration of supplementation.

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