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ORIGINAL

Effect of estrogen and insulin sensitivity due to exercise training with dual intensities in female rats with estradiol valerate-induced PCOS

Efecto de la sensibilidad a los estrógenos y la insulina debido al entrenamiento con ejercicios de intensidad dual en ratas hembras con SOP inducido por valerato de Estradiol

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Abstract

Background: Exercise training can improve polycystic ovary syndrome (PCOS). However, the effect of various intensities of exercise training is unclear, hence the purpose of this study was to evaluate the effect of exercise training with dual intensities on estrogen and insulin sensitivity in female rats with estradiol valerate-induced PCOS.
Methods: In this semi experimental study, forty adult (Wistar rats weight: 180±20 gr) after induction of PCOS, were divided into four groups (n=10):healthy control (HC), polycystic control (PC), low intensity training (LI) and moderate intensity training (MI) groups. Exercise training consisted of eight-week exercise with 50-55% maximal oxygen consumption (20m/min speed) and 70-75% maximal oxygen consumption (28m/min speed), 3 days a week for 60 minutes. Data analysis was performed using SPSS software (version 16.0, SPSS). One-was ANOVA test was used for data analysis. The level of significance was set at $p<0.05$.
Results: Significant difference was observed between glucose concentration of MI and PC (p=0.039), also there was significant difference between LI and PC (p=0.005). Insulin significantly redreased in MI compared to PC (p=0.002). Also, significant difference was found between LI and PC (p=0.005).
Conclusion: It seems that exercise training with low and moderate intensity may reduce insulin and glucose level. Low intensity may improve insulin sensitivity, and can be considered as a non-pharmacological treatment method to regulate glucose hemostasis in polycystic ovary syndrome patients.

Keywords: Polycystic ovary syndrome, exercise training, insulin sensitivity, estrogen.

Resumen

Antecedentes: El entrenamiento con ejercicios puede mejorar el síndrome de ovario poliquístico (SOP). Sin embargo, el efecto de varias intensidades de entrenamiento no está claro, por lo tanto, el propósito de este estudio fue evaluar el efecto del entrenamiento con intensidades duales en los estrógenos y la sensibilidad a la insulina en ratas hembras con SOP inducido por valerato de estradiol.
Métodos: En este estudio semiexperimental, cuarenta ratas adultas (peso de las ratas Wistar: 180±20 gr) tras la inducción del SOP, fueron divididas en cuatro grupos (n=10): control sano (HC), control poliquístico (PC), entrenamiento de baja intensidad (LI) y entrenamiento de intensidad moderada (MI). El entrenamiento consistía en ocho semanas de ejercicio con un 50-55% de consumo máximo de oxígeno (velocidad de 20 m/min) y un 70-75% de consumo máximo de oxígeno (28m/min de velocidad), 3 días a la semana durante 60 minutos. El análisis de los datos se realizó con el software SPSS (versión 16.0, SPSS). Para el análisis de los datos se utilizó la prueba de ANOVA de una variable. El nivel de significación se fijó en $p<0,05$.
Resultados: Se observó una diferencia significativa entre la concentración de glucosa de MI y PC (p=0,039), también hubo una diferencia significativa entre LI y PC (p=0,005). La insulina se redujo significativamente en el IM en comparación con el CP (p=0,002). También se encontró una diferencia significativa entre el IM y el CP (p=0,005).
Conclusiones: Parece que el entrenamiento con ejercicios de baja y moderada intensidad puede reducir el nivel de insulina y glucosa. La baja intensidad puede mejorar la sensibilidad a la insulina, y puede considerarse como un método de tratamiento no farmacológico para regular la hemostasia de la glucosa en pacientes con síndrome de ovario poliquístico.

Palabras clave: Síndrome de ovario poliquístico, entrenamiento con ejercicios, sensibilidad a la insulina, estrógenos.

Introduction

It is obvious that infertility is one of the main problems that have been increased since 1955 and also 10%-15% of individuals are suffering from it. Polycystic ovary syndrome (PCOS) is one of the causes of infertility .PCOS is the most common endocrine abnormality in premenopausal women. It seems that genetic etiology plays an important role in this disorder[1]. The most prevalent characteristic of PCOS are irregular menstrual cycles, imbalance of sex hormones and polycystic ovarian morphology. It is reported that metabolic disorders, such as insulin resistance is associated with PCOS. Severity and incidence of PCOS is characterized by life style[2]. So the result frightening the life is consists of Breast cancer risk, cardio vascular disease and type two diabetes[1]. Diabetes mellitus is also associated with central obesity and increased level of incomplete insulin functions of pancreatic cells and increased level of leptin[3, 4]. In accordance to previous researches 50-70 percent of females have some level of PCO and resistance to insulin. This resistance maybe due to obesity or hyperandrogenism[5]. Previous studies reported that PCOS is not the malfunction of some specific part of body because recent findings show that hormonal changes might play role in this syndrome such as increased level of androgens, 17 alpha hydroxyprogesterone and estrogen[6]. Also increased serum level of LH is prevalent among female with PCOS. The increased release of LH is associated with free Estradiol and estrogen[7]. Nevertheless, there is a specific relationship between serum level of estrogen and biochemical index of insulin resistance in PCOS females. Scientists believe that regular exercise training is a safe method of treatment without regarding clinical actions[2]. Jedel et al. (2011) conducted a research to investigate the effect of exercise training on hyperandrogenism. 84 PCOS females participated in a period of long term training and finally results revealed that estrogen and androgen reduced due to exercise[8]. Also literatures studying PCOS showed that exercise training change level of sexual hormone such as estrogen without regarding weight change or insulin sensitivity[9]. In addition to hormonal disorders ,PCOS are faced with metabolic disturbance such as insulin resistance[4]. Its mechanism in PCOS is different and about 60 percent of PCOS patients are struggling with insulin resistance too. It is reported that the mean number of PCOS patient are more than healthy people especially in the obese individuals[10]. Also exercise training is recommended for prevention and treatment of these patients with insulin sensitivity. Doing exercise training regularly accompanied with dietary regimen are those elements that lead to weight reduction and hormonal adjustment in females[11]. In this field, exercise training ensue reduction in insulin levels and improvement in glucose usage and insulin sensitivity which could decrease body fat[12, 13]. Researchers believe that not high intensity but probably regular exercise is a natural healthy cure. In this direction, Benrick et al. (2013) reported that exercise training led to significant reduction of glucose comparing to PCOS control[14]. Qui and et al. 2009 investigated the effect of training on insulin resistance in 20 wistar rats undertaken PCOS. The rats swam 120 min for 2 weeks. The final result showed that serum level of insulin decreased after exercise training but no significant change was observed in plasma glucose compared to control group[15]. Since there is a contradiction about the intensity of exercise training and the effect of exercise intensity in PCOS have not been clearly determined regarding the influence of exercise training in improvement of hormonal imbalance and glucose hemostasis, in the aim of the present study was to investigate changes of estrogen and insulin sensitivity due to exercise training with dual intensities in female rats with estradiol valerate-induced PCOS.

Material and methods

Animals

Forty female Wistar rats were (180 ± 20 g) were selected and kept in animal house. They had every 2 to 3 consecutive estrous cycles during 12 to14 day. Their cages were clarified three times per week with alcohol and adequate water in appropriate container was catered.

Approval

This research was confirmed by ethical committee of Jahrom University of Medical Sciences and morality was considered.

Induction of PCOS

A variety of hormonal and non-hormonal methods to PCO phenotype, consisting long-term use of light, testosterone, dehydroepiandrosterone (DHT), adrenocorticotropic and estradiol valerate. In this study, we utilized estradiol Valerate. 30 female rats were randomly singled out from forty. 4 mg of estradiol validate dissolved in 0.2 mg of Sesame oil was injected (IM) in thigh area[16].

Protocol design

Initially, subjects were divided into control (n = 10) and polycystic (n = 30) groups that were overtaken PCOS by intramuscular estradiol valerate injection following 60 days. The PCOS subjects were split into 3 groups of polycystic control (PC) (n =10), low-intensity exercise (LI) (n =10) and moderate intensity exercise (MI) (n =10). Training was executed 1 h/day, 6 days/week for eight weeks. Moderate intensity: (28 m/min-70%-75%VO2Max), and Low intensity (20 m/min-50%-55%VO2Max) running were performed at 0 slope.

Exercise training protocol

The LI and MI were trained on a rodent motor-driven treadmill at a 0° slope. For induction of adaptation, rats

exercised at Treadmill velocity of 10 m/min for 15min during the 1st work of training. The treadmill velocity and exercise duration elevated gradually until the animals ran for 1 h/day during the 2nd and 3rd work of training. The treadmill speed and exercise duration were constant. Frequency (6d/work) and duration (1 h/d) were constant during the study.

Vaginal smears and blood sampling

microscopic analysis of the predominant cell types qualified estrus cycle stage obtained through daily vaginal smears[16] Vaginal smear test was done in two month for certitude of PCOS induction within subjects. After the test, we selected those having 2 or 3 regular estrous cycle within 12-14 days. Blood sample was directly taken from their heart through a 5 cc syringe following 32 hours after the last exercise bout. After isolation of blood serum, dependent variables were assessed.

Blood collection and tissue preparation

To minimize the effect of last exercise session, the subjects were finally anesthetized with diethyl ether and sodium pentobarbital (50 mg/kg, intraperitoneal injection) after a12-h fasting and 32 h after the last exercise session and their blood was obtained from the heart. Tubes containing plasma sample aliquots were kept frozen at -80°C until being analyzed[16].

Measurement

Estrogen was assessed by ELISA kit (monobind e2).Insulin sensitivity was calculated by quantitative insulin sensitivity check index (*QUICKI*) formula: 1 / (log (fasting insulin μU/mL) + log (fasting glucose mg/dL)). ELISA kits specific for the rat were used to determine plasma insulin (BioVendor Shibayagi, Japan), and serum glucose was measured via spectrophotometry method (spectrophotometer wavelength was adjusted in 650 nm).

Analyzing method

A Shapiro-wilk test was applied to determine normality of distribution which was found to be normally distributed. A one-way analysis of variance (ANOVA) was performed to determine the differences in a parameter among the groups. Significant differences were identified using a least significant difference (Bonferroni) post-hoc test.

Results

Table I shows the indicates biochemical blood parameters in groups (mean±S.D).

Table I: Indicates biochemical blood parameters in groups (mean±S.D).

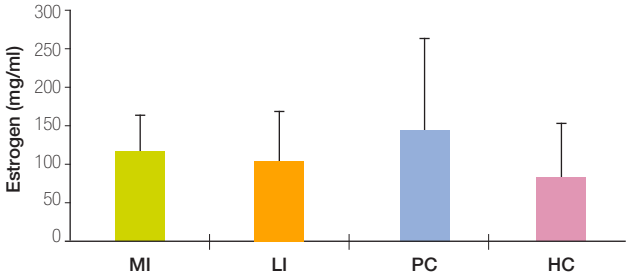
Groups	Estrogen (ng/ml)	Glucose (mg/dl)	Insulin (μU/ml)	Insulin sensitivity
MI	119.01±50.56	157.08±31.7 *	0.56±0.25 *	0.54±0.06
LI	104.84±66.22	145.02±22.8 *	0.41±0.3 *	0.6±0.14 *
PC	144.79±121.53	203.2±52.9	1.5±0.75	0.41±0.04
HC	86.98±67.99	94.7±17.5	0.65±0.52	0.63±0.1

Values are mean ± SD * p < 0.05 vs. PC

Estrogen

No significant difference was observed between MI (119.01±50.56) and PC (144.79±121.53) also, there was no statistical change between LI (104.84±66.22) and PC, although both MI and LI showed reduction comparing to PC. No significance reduction was in LI compared to PC. Comparing HI and PC showed a significant difference, (**Figure 1**).

Figure 1: Glucose level of groups after 8 week.

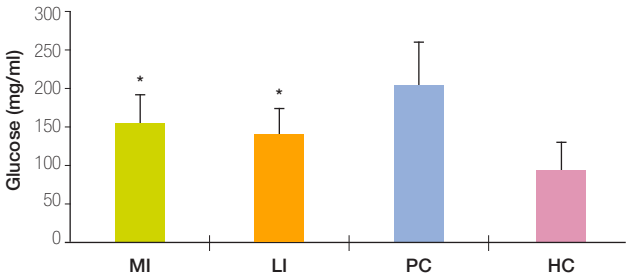


*significant comparing to PC (p<0.05)

Glucose

According to finding illustrated in the **table I**, significant reduction was observed between glucose concentration of MI(157.08±31.7) and PC(203.2±52.9) (p=0.039), also there was significant decrease between LI (145.02±22.8) and PC (p=0.005) but no significant change was seen between MI and LI, (**Figure 2**).

Figure 2: Glucose level of groups after 8 week.

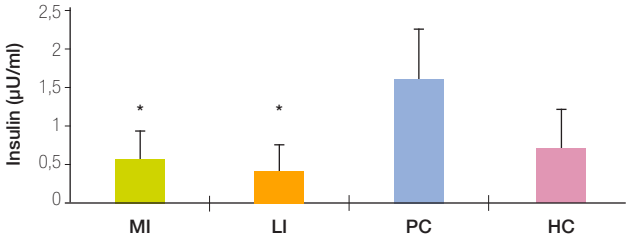


*significant comparing to PC (p<0.05)

Insulin

After 8 week exercise training, serum level of insulin did significantly change in MI (0.41±0.3) comparing to PC (1.5±0.75) (p=0.002). Also statistically significant reduction was observed between LI (0.566±0.25) and PC (p=0.00). There was no significant change between MI and LI, (**Figure 3**).

Figure 3: Insulin level of groups after 8 week.

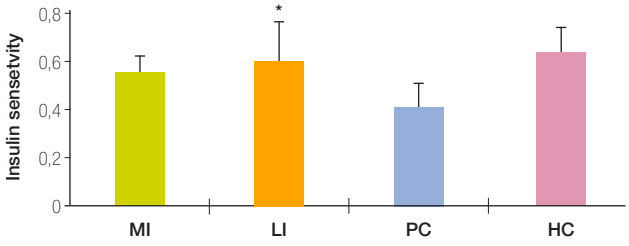


*significant comparing to PC (p<0.05)

Insulin sensitivity

Base on **table I**, a significant elevation was observed between LI (0.6±0.14) and PC (0.41±0.04), (p=0.003), but no significant change was between MI (0.54±0.06) and PC, Also no statistical difference was found between MI and LI, (**Figure 4**).

Figure 4: Insulin level of groups after 8 week.



*significant comparing to PC (p<0.05)

Discussion

The aim of this study was to examine the effect of eight weeks exercise training with different intensities on levels of estrogen and insulin, glucose and insulin sensitivity due to exercise training with dual intensities in female rats with polycystic ovary syndrome. The results of the present investigation showed that the eight-week exercise training induced a significant change in glucose level in low intensity group in comparison to PC (**Figure 2**). This finding was supported by previous researches[17,18]. However, there is a controversy in the results obtained by Holm and colleagues (2010)[19]. Benrick et al. (2013) conducted a research on rats with polycystic ovary syndrome, and finally concluded that exercise training leads to the a significant declined in glucose level in these rats, moreover, exercise can lead to decrease in Insulin resistance in rats with polycystic disease[18]. Qiu et al. (2009)[15] examined the effect of exercise on improvement of Insulin resistance in 21 day wistar rats with polycystic disease for 120 minutes of swimming per day for two weeks, concluded that testosterone and insulin levels in practice was significantly lower than control group. But, they did not observe the significant differences among groups in glucose and progesterone values. Among the mechanisms that lead to decrease glucose level after aerobic exercise, included the increased of post-insulin recipient's message[20]; increase in expression of transfer protein of glucose, GIULT-4[21]; increasing the activity of glycogen synthase hexokinase[22]; Reduction of secretion and elevation of clarification of free fatty acids; glucose uptake from the blood to muscle can be due to the increase of the muscle capillaries and alteration in muscle structure in order to increase intake of glucose[23]. Therefore, one of the methods of reducing insulin resistance and risk of cardiac disease, type II diabetes, is aerobic exercise particularly in overweight and PCOS patients. One of the limitation of this research was that these models do not reproduce exactly what happens in the real syndrome which may affect our findings.

In non-inflammation situation, TNF-alpha is derived from adipose tissue and its plasma level is associated with body fat mass. TNF-a opposites by insulin Message forming through signaling reduction by means of phosphorylation serine[24]. In this research it was revealed that insulin level was significantly decreased -%73 in LI and -%62 in MI comparing to PC. This finding was supported by previous investigations[17, 18]. However, there is a controversy in the results obtained by Holm and colleagues (2010)[19]. Fasting hyperinsulinemia is abundant in obese PCOS patients and this is, in part, secondary to increased basal insulin secretion rates[25]. Insulin responses to an oral glucose load are elevated in lean and obese PCOS patients, but acute insulin responses to an intravenous glucose load (AIRg), first-phase insulin secretion, are similar to weight-matched control women. When the relationship between insulin secretion and sensitivity is examined lean and obese PCOS women fall below the relationship in weight-matched control women, and the disposition index is significantly decreased by PCOS as well as by obesity[26]. In this study, training period was considered as 8-weeks. In addition, the training was performed with two intensities (low and moderate) for 60 minutes. Furthermore, recently some studies showed that training with low to moderate-intensity training can cause weight loss and body fat percent loss and increased fat oxidation[27, 28], Hence, the low intensity exercise, is a common type exercise in researches due to the decline of weight and considered as appropriate intensity. In the study, we observed no significant change in estrogen level in PCOS groups. Gonadotropin is the glycoprotein that is released in response to gonadotropin-releasing hormone (GnRH) from the pituitary. The sinus mood release of GnRH causes production of estrogen concentrations. Response of estrogen levels to exercise depends on menstrual cycle which complicates sexual hormones response to physical activity. Another aspect of this complexity are reduction of body fat that can induce some changes in estrogen secretion[29]. On the other hand, physical activity can increase hormone of beta endorphin which cause reduction in GnRH, followed by decrease in estrogen levels[30]. The results obtained from this study imply the role of intensity/volume of training on insulin sensitivity which are considered as crucial variables that can determine healing process of PCOS[31, 32].

Conclusion

As a result, according to this result, Low and moderate intensity exercise may be effective in reduction of glucose and insulin level. Low intensity could elevate insulin sensitivity, and could be considered as a non-pharmacological treatment method to regulate glucose hemostasis in polycystic ovary syndrome patients. Although, due to the lack of research evaluating the influence of different exercise intensities on patients with Polycystic syndrome future studies are recommended for more clarification.

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