

Original Article

# The effects of moderate treadmill and running wheel exercises on oxidative stress in female rats with steroid-induced polycystic ovaries

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## Abstract

**Introduction:** Polycystic ovary syndrome (PCOS) is one of the most common endocrinological pathologies in women during their reproductive years with ovulatory dysfunction, abdominal obesity, hyperandrogenism and insulin resistance. The aim of the present research was to evaluate the total antioxidant capacity (TAC), total oxidant status (TOS), free testosterone, ovarian morphology and estrous cyclicity in the estradiol valerate (EV)-induced PCOS rat model and the effect of treadmill and running wheel exercises on these parameters.

**Methods:** Fifty female Wistar rats were randomly selected ( $220 \pm 20$  g). They had every 2 to 3 consecutive estrous cycles during 12 to 14 days. The first two groups were divided into control (n=10) and polycystic (n=40) that were induced PCOS by EV injection after 60 days. The polycystic groups were divided into three groups (n=10 in each group) PCOS, experiment group with treadmill exercise (running for 28 m/min at 60 min/day) and experiment group with running wheel exercise (running daily for 4 hours) for 8 weeks.

**Results:** The PCOS rats had significantly higher testosterone, TOS and lower TAC than control. Eight weeks of treadmill and running wheel exercise significantly increased serum levels of TAC (just for treadmill exercise) and decreased level of TOS and T (just for treadmill exercise) in EV-induced PCOS rats compared to PCOS group. Ovarian morphology and estrous cycle was almost normalized in the PCOS exercise (treadmill and running wheel) groups.

**Conclusion:** The present study demonstrate EV-induced PCOS in rats is associated with an increased oxidative stress and this increase can be returned to normal levels by exercise.

## Keywords:

Polycystic ovarian syndrome;  
Oxidative stress;  
Running wheel exercise;  
Treadmill exercise

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## Introduction

Polycystic ovary syndrome (PCOS) is the commonest endocrine disorder of reproductive-age

women presenting in ~7% of this population. It is often associated with the presence of polycystic ovaries, oligo and/or anovulation, biochemical (elevated androgens), clinical (hirsutism and/or acne) hyper androgenism and metabolic disorders

including insulin resistance, obesity and diabetes. The true pathogenesis of this enigmatic syndrome appears to be multifactorial and polygenic and its underlying basis remains unclear (Erickson et al., 1979; Erickson and Yen, 1984; Clark et al., 1995; Azziz et al., 2004; Burghardt et al., 2004; van der Spuy and Dyer, 2004; Buggs and Rosenfield, 2005; Boomsma et al., 2006; Brassard et al., 2008; Callard et al., 2011; Diane et al., 2015).

Hyperandrogenism is the central feature of PCOS and a chronic androgen excess of ovarian and/or adrenal origin can result in widespread biochemical feature in PCOS women (Rosenfield, 1997; Jonard and Dewailly, 2004). Intraovarian hyperandrogenism may be causatively linked with anovulation in PCOS and impair folliculogenesis in a binary way, in part by stimulating the growth of small follicles and in part by hindering follicular maturation towards the dominant stage (Franks, 2006).

There are accumulating evidence that support the speculation that PCOS is accompanied to increased oxidative stress and systemic inflammation (Sabuncu et al., 2001; Orzio Jr et al., 2005). Oxidative stress occurs when the balance between reactive oxygen species (ROS) and antioxidants is tipped towards overabundance of ROS. Reproductive cells and tissues will remain steady only when antioxidants and oxidant status are in balance. Oxidative stress, which is commonly known to be current in women with PCOS regardless of whether they are lean or have metabolic abnormalities. It might also contribute to PCOS and its metabolic associations (Sabuncu et al., 2001; Agarwal et al., 2005).

The effects of hyperandrogenemia on oxidant and antioxidant status in women with PCOS is not clear. However, *in vitro* studies have proved that oxidative stress correlates with androgen-producing ovarian steroidogenic enzymes, while antioxidants such as statins suppress these enzymes (Piotrowski et al., 2005).

Oxidative stress is a process subject to PCOS and several factors including physical activity, which seem to modulate oxidative stress. Lifestyle interventions focusing on diet and exercise are considered first-line treatments to target reductions in total body weight and treatment in PCOS (Thomson et al., 2011; Moran et al., 2013).

Overall, several of literature describes an

association of physical activity and general well-being including preservation against the development of heart disease, diabetes and improvement in mood in human (Warburton et al., 2006). In animals, however, data are rare and opposite mainly due to use of different exercise models (Burghardt et al., 2004; Ang and Gomez-Pinilla, 2007; Leasure and Jones, 2008). In fact, several studies have been stated exhaustive exercise elevates ROS, results in oxidative damage (Aguiló et al., 2005; Rosa et al., 2007); while moderate exercise has been reported to arouse adaptation of brain antioxidant system by increasing its resistance to oxidative stress (Somani and Husain, 1997; Radak et al., 2001).

In PCOS women, exercise appears to have advantageous effects with reports of betterments in fitness, body composition, fasting insulin, IR, menstrual cyclicity, ovulation, self-esteem, quality of life scores and depression. Despite well-established profits of exercise training and its recommendation as a cornerstone of PCOS management, few well-controlled studies have assessed the advantages of exercise training in PCOS (Thomson et al., 2011).

Although, there are some investigations on exercise effect on PCOS (Thomson et al., 2011), however, there is no information about the effects of treadmill exercise (8 weeks) on moderate intensity (28 m/min, 0% grade) and voluntary exercise (running wheel exercise) on oxidative stress. Therefore, the purpose of present study is to investigate the effects of exercise on weight changes, free testosterone, total oxidant and antioxidant status levels in the estradiol valerate (EV)-induced PCOS rat model.

## Materials and methods

### Animals and protocols

The experiments were carried out on female adult Wistar rats (n=50, age 90 days and body weight 220-230 g) were purchased from Shahid Beheshti University (Tehran, Iran), kept under controlled conditions (12 h-12 h light- dark cycles and controlled temperature of 21–22 °C, relative humidity 55– 65%) with free access to food and water. All procedures for the maintenance and use of experimental animals were approved by the ethics committee of Neuroscience Research Center of Shahid Beheshti University of Medical Sciences (Tehran, Iran).

Rats were randomly divided into two groups: the EV-induced PCOS rats (n=40) which were given a single dose intramuscular injection of estradiol valerate, 4 mg/kg body weight dissolved in 0.2 ml olive oil, to induce well-defined PCOS (Brawer et al., 1986) and control (n=10) which were injected with proper volume of olive oil alone. After 8 weeks of EV injection, rats in PCOS group were randomly subdivided into two treatment of ten animals each: PCOS, PCOS plus exercise on treadmill (P+ExT, n=10) and PCOS plus exercise on running wheel (P+ExR, n=10) groups. The treatment lasted 8 weeks. The rats in control and PCOS groups did not participate in exercise. Eight weeks following the intervention, the estrous cyclicity was measured daily for 21 consecutive days.

#### **Determination of estrous cycle (vaginal smear)**

For 10 days before the experiment, estrous cyclicity was monitored by microscopic analysis obtained via vaginal smear to determine the stage of estrous cycle. The rat estrous cycle (pro-estrus, estrus, metestrus, diestrus) usually lasts for 4 days (Witchel and Tena-Sempere, 2013). At the beginning of experiment, all rats showed regular cycles. Smears obtained by vaginal washing were dyed with methylthioninium chloride and then analyzed under a microscope for the predominant cell type in vaginal smears. After vaginal smear test, we used animals having 2 or 3 regular estrous cycle.

The proportions of leucocytes, epithelial and cornified cells were determined a characteristic manner during the different stages of the estrous cycle. Pro-estrus was characterized as primarily nucleated epithelial cells, estrus as an abundance of anucleated cornified cells, metestrus as presence of leukocytes, cornified and nucleated epithelial cells and diestrus as predominance of leukocytes and nucleated epithelial cells (Shi and Vine, 2012). After injection of estradiol valerat, vaginal smear test was taken within 60 days for reassurance of induction of PCOS in experiment groups.

#### **Physical exercise**

##### **Chronic voluntary exercise (running wheel):**

Rats in the PCOS running wheel exercise group were placed in the wheel running apparatus for 4 h/day with free access to water but no food at this time. This exercise was voluntary in nature compared to

forced treadmill exercise. Running wheel distance was recorded daily and body weight was measured two times a week.

##### **Physical exercise on treadmill:**

Treadmill training began with familiarization of rats for five days consisted of walking on a treadmill for 10 min at 5 m/min. Treadmill training was performed between 8:00 and 12:00. The grid was not changed during exercise.

Training group was given exercise training for five days/week for 8 weeks as described previously. Every session started with 12 m/min at 5 minutes for preparing rats for main training. In the first week, rats were trained on the treadmill at the speed of 15 m/min and the running time was 10 min/day. In the second week the speed was increased to 15 m/min and the duration to 30 min/day. In the third week, the speed was increased to 20 m/min, while the duration was increased to 45 min/day. In the fourth week, the speed remained 28 m/min and the duration increased to 60 min/day (Table 1). In the last 4 weeks the speed and duration remained constant. This condition corresponded to a moderate intensity of about of 65-70% of maximal oxygen consumption (Thirunavukkarasu et al., 2003).

##### **Ovarian morphological analysis**

The animals were not exercised for 24 h prior to sacrifice. When rats were sacrificed, Ovaries of control, PCOS and exercised PCOS groups were removed (n=6 rats in each group), cleaned from connective tissue and fixed in phosphate-buffered saline-formalin at room temperature for 3 days followed by immersion in xylene and embedded in paraffin. The ovaries were longitudinally and serially sectioned at 4  $\mu$ m from the center, serial sections were mounted on 3 slides, deparaffinized with xylene, hydrated with successive decreasing concentrations of ethanol, and then stained with hematoxylin and eosin (Wu et al., 2010).

The section with the largest area was chosen for analysis to count the numbers of preantral, antral, atretic follicles and corpora lutea by counting five representative sections per ovary at least 30  $\mu$ m apart. All sections were photographed with a camera (Olympus BX51, Japan) at  $\times$ 40 and  $\times$ 100 magnifications. The identification of types of ovarian follicles were assessed and counted by two persons

(to avoid duplicate counting).

### Blood samples

All rats were fasted overnight and twenty-four hours after the last exercise session (8th week) animals were anesthetized with ketamine and xylazine and then blood samples were collected in a volume of 0.5 ml. Heparin was added to the samples to prevent clotting. Blood samples were immediately centrifuged at 3000 g for 20 min and plasma was frozen ( $-20^{\circ}\text{C}$ ) until further analysis. Rat testosterone ELISA kits were used to measure levels of testosterone (T).

ZellBio GmbH TAC (total antioxidant capacity) and TOS (total oxidant status) assay kits have been

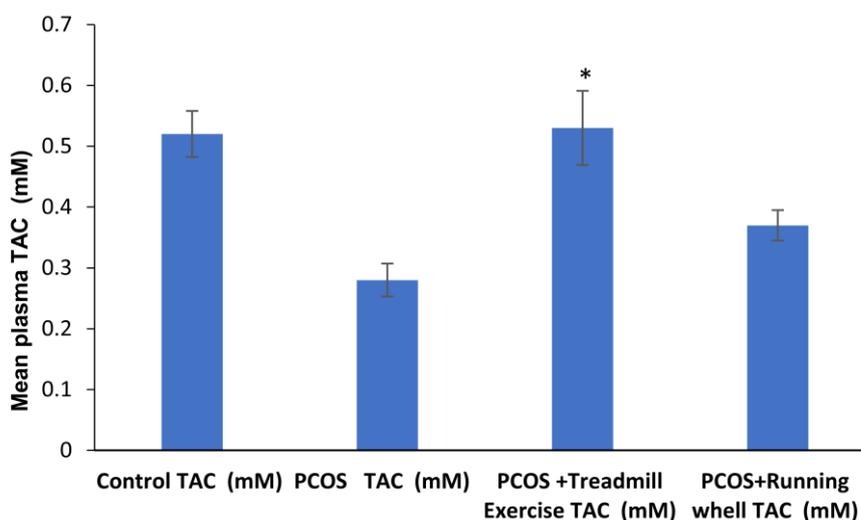
utilized for assessment of TAC and TOS serums in colorimetrically at 490 nm and at 560 nm respectively determined by ELISA.

### Statistical analyses

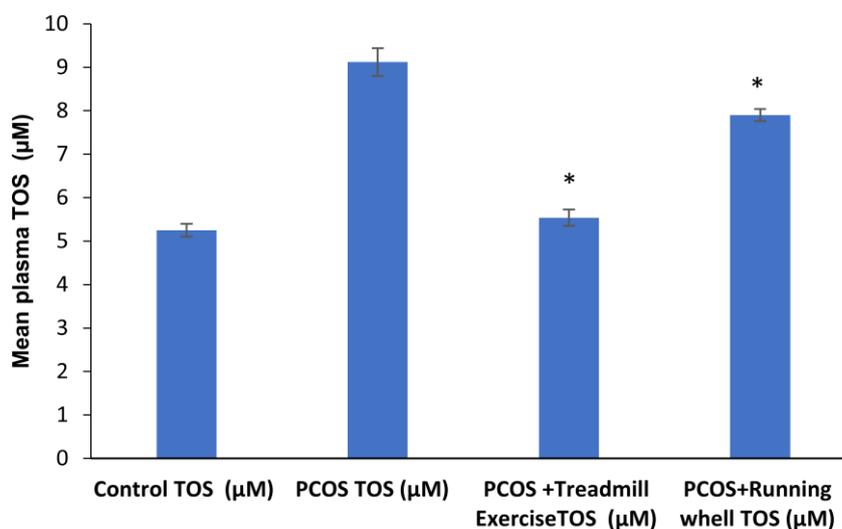
Results are presented as mean  $\pm$  SEM. Data were analyzed by one-way ANOVA test followed by post hoc Tukey's test using the SPSS software (version 16). In all cases, significant difference was defined as  $P < 0.05$ .

## Results

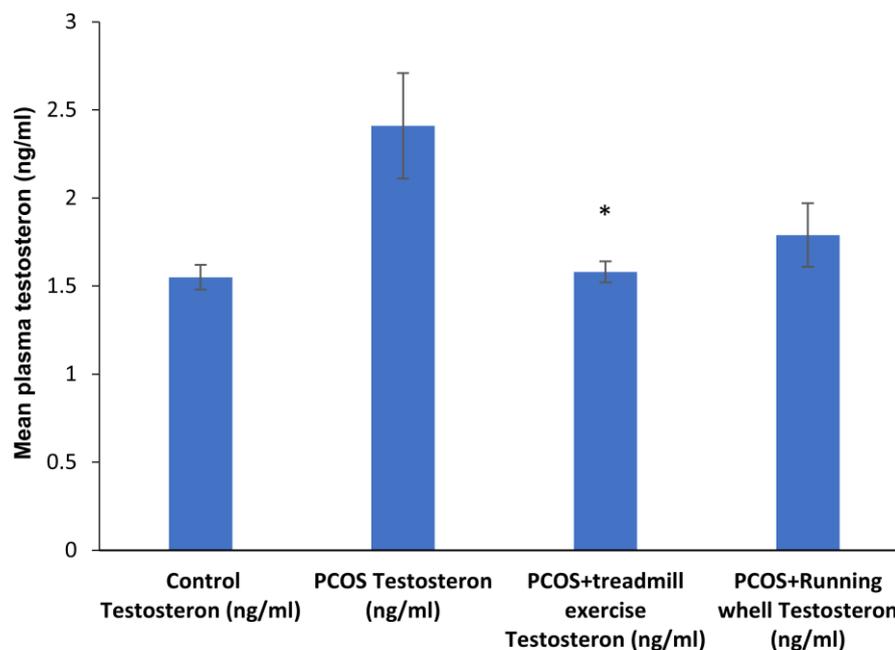
### The effects of exercise on serum testosterone,



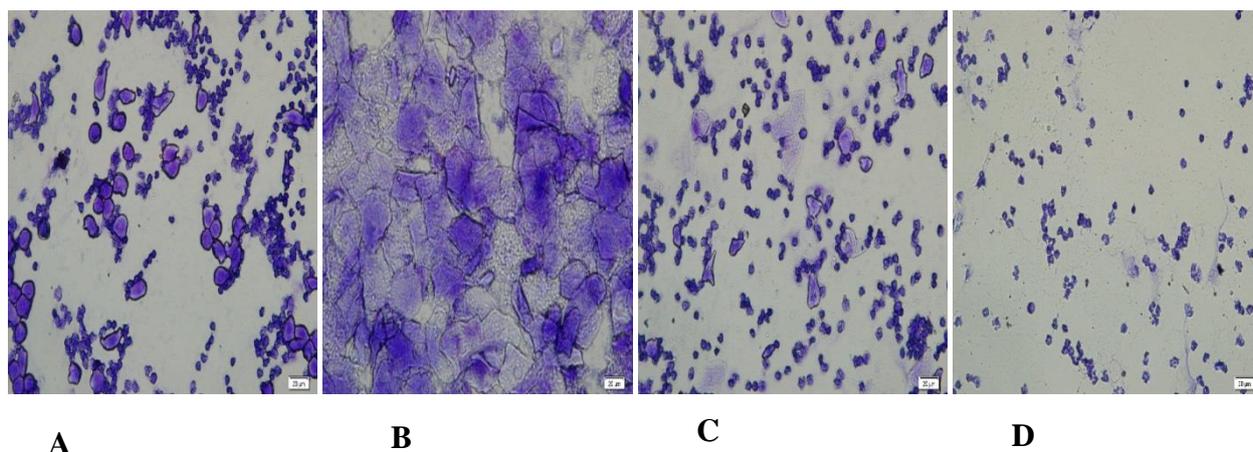
**Fig.1.** Effect of exercise (running wheel and treadmill) on mean plasma TAC concentration in EV-induced PCOS rats. Values are mean  $\pm$  SD \* $P < 0.05$  versus PCOS by Tukey post-hoc test.



**Fig.2.** Effect of exercise (running wheel and treadmill) on mean plasma TOS concentration in EV-induced PCOS rats. Values are mean  $\pm$  SD \* $P < 0.05$  versus PCOS by Tukey post-hoc test.



**Fig.3.** Effect of exercise (running wheel and treadmill) on mean plasma testosterone concentration in EV-induced PCOS rats. Values are mean  $\pm$  SD \* $P$ <0.05 versus PCOS by Tukey post-hoc test.



**Fig.4.** Cell types of vaginal smear. A: pro-estrus, loaded mainly with epithelial cells; B: estrus, loaded mainly with keratinocytes; C: metoestrus, displaying epithelial cells, keratinocytes, and leukocytes; D: diestrus, displaying full of leukocytes (magnification  $\times$ 100)

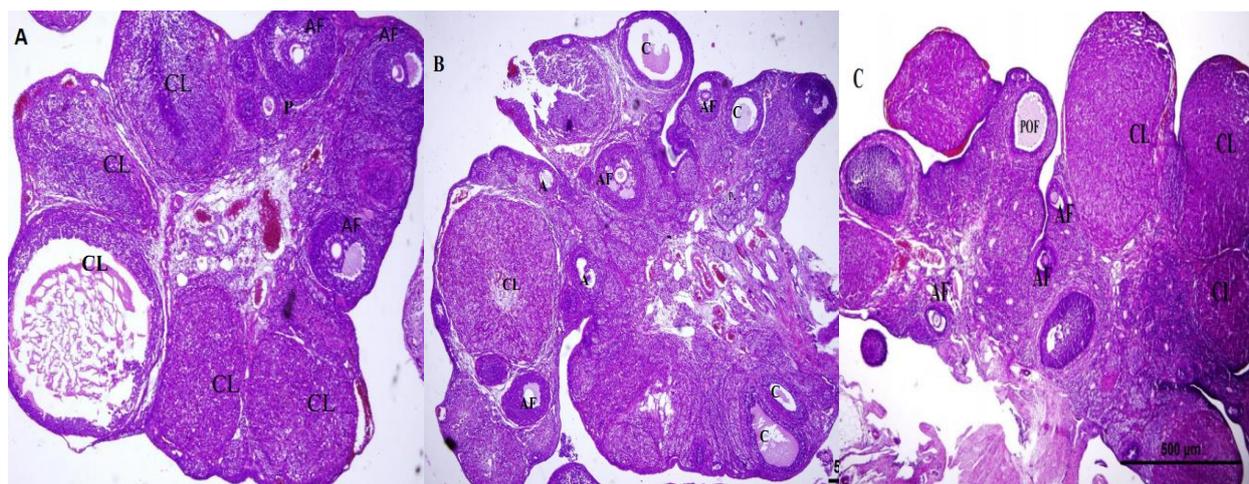
#### total serum antioxidant and total serum oxidative stress

Results of comparison of T, TAC and TOS between groups are illustrated in Fig. 1, 2 and 3. Eight weeks of treadmill exercise (P+ExT) significantly increased serum levels of TAC and decreased levels of TOS and T in EV-induced PCOS rats compared to PCOS group and similar to control group. In comparison with PCOS, TOS were significantly decreased in running wheel exercise (P+ExR) group, got close to control group. However, the serum levels of testosterone and TAC was not significantly decreased and increased in

this group, respectively.

#### Estrous cycle

The vaginal smears were monitored during whole experiment. The regular estrous cycle of 4–5 days was observed in control. However, EV-induced PCOS rats had irregular cycles and there were many white cells, less epithelial cells and keratinocytes in vaginal smears of PCOS rats, indicating that PCOS rats had longer and irregular estrous cycles compared with control (Fig. 4). After 8 weeks, the rats in P+ExT and P+ExR groups restored to normal



**Fig.5.** Ovarian morphology of rats from three groups (×40). A: control. Several antral follicles (AF), corpora lutea (CL) and preantral follicles (p) are shown. B: showing atretic follicles (AF) and cystic follicles (c) in EV-induced PCOS rats group. C: PCOS plus exercise. Antral follicles (A), pre-ovulatory follicle (POF) and a bit of corpora lutea (CL) are shown in the section.

**Table 1:** The program of treadmill exercise.

Week	Duration (min)	Speed (m/min)
1	10	15
2	30	15
3	45	20
4	60	28
5	60	28
6	60	28
7	60	28
8	60	28

**Table 2:** Effects of treadmill and running wheel exercises on body weight and ovarian weight. Significant differences are indicated by letters, a: compared to PCOS group, b: compared to control group. Data present mean ±SEM, *P*<0.05, n=10 in each group.

Variable	Control	PCOS	P+ExT	P+ExR
Body weight (g)	239.8±2.48	255±2.99	210.3±1.87a	213.7±1.71a
Ovary weight (mg)	84.5±1.33	40.3±1.54b	42.8±0.9b	40.0±1.18b

estrous cycles improved with more days in pro-estrus and estrus and fewer in diestrus.

**Effect of exercise on body weight**

The body weights of the rats are presented in Table 2. At the end of the experiment, the body weights of the exercise groups (P+ExR and P+ExT) were significantly lower comparing with PCOS group. The body weight of PCOS group increased significantly compared to control and exercise groups.

There was a significant effect of EV injection on the weight of the ovaries, whereas there was no effect of exercise. The ovaries of both the PCOS and the PCOS exercise groups weighed less than the ovaries of the control and exercise groups.

**Ovarian morphology analysis**

The ovaries in the control group were normal with follicles in different stages of development and corpora lutea. Follicular atresia and multiple cysts

were observed in the ovaries of PCOS group. The number of antral follicles in the ovaries of PCOS group was decreased compared with control rats. The numbers of corpora lutea and preovulatory follicles in PCOS group were significantly less than those in control and PCOS exercise groups (P+ExR and P+ExT). The morphology of the ovaries of the PCOS rats with eight weeks of treadmill and voluntary exercise (the PCOS exercise group) was almost normal (Fig. 5).

## Discussion

This study demonstrated that 8 weeks running wheel and treadmill exercise may cause weight reduction and modify free testosterone, TOS and TAS in EV-induced PCOS rats. PCOS is a complex endocrine and metabolic disorder associated with anovulation, infertility and hyperandrogenism in women during their reproductive years, exhibiting a wide spectrum of clinical manifestations.

Hypersecretion of androgens is the primary manifestation and the main pathophysiological feature of PCOS. Chronic androgen excess in PCOS is associated with disorder in growing of follicles. It can be considered that increased androgen can contribute to increase oxidative stress in PCOS.

We observed the PCOS rat injected with EV showed both ovarian and metabolic characteristic as follows: (1) hyperandrogenism (2) disrupted estrous cycle (persistent estrous periods) observed by serial vaginal smears (3) PCO-like changes including recruitment of preantral follicles and the appearance of cystic follicles.

Research in recent years indicated that oxidative stress in PCOS women is higher in comparison to control group that causes metabolic syndrome and type 2 diabetes. In this study, consistent with previous studies, TOS was increased and TAC was lowered in EV-induced PCOS rats compared to control group.

In 2013, Hillai et al., have shown that prolidase enzyme activity and oxidative stress are increased in PCOS woman. Significant changes in oxidative stress, insulin resistance, and expression of p47 phox protein, mediating superoxide anion production, have been also reported in PCOS women (González et al., 2012). Duleba et al. (2004), have shown that increases in androgen synthesis and theca cell

development observed in PCOS are caused by oxidative stress and free radicals.

Multi factors play role in decreasing oxidative stress. One of these factors is participating in regular exercise. Participation in regular exercise is key factor for weight maintenance, improvement of menstrual cycle and ovulation in 50% of PCOS women. Studies have shown that exercise had many benefits to improvement of PCOS. A decrease in body weight gain was indicative of beneficial training adaptations in rats (Hoeger, 2008; Thomson et al., 2011; González et al., 2012).

Intervention studies to see if exercise can improve oxidative stress in women with PCOS are lacking (Harrison et al., 2011; Jedel et al., 2011; Harrison et al., 2012; Haqq et al., 2014), as are studies in animal models.

Consistent with the reports by other researchers, we found that the body weight was lower in exercise PCOS group (running wheel and treadmill) compared to EV-induced PCOS rats. The result of this study have shown that treadmill exercise can favorably modulate oxidative stress via increased total antioxidant and decreased total oxidative stress in EV-induced PCOS rats compared to PCOS group. Running wheel exercise group had significant lower TOS than PCOS group. However, TAS and testosterone levels were not significantly higher and lower compared with PCOS group, respectively.

Group et al., (2002) have recently demonstrated that 24 -weeks training including 30 minute bicycling three times a week in PCOS women resulted in a significant decrease in fasting insulin and insulin resistance. Lampman and Schteingart reported that exercise plus diet significantly changed the improvement of insulin sensitivity. Therefore, moderate exercise training had an improvement in insulin sensitivity (1991).

Other researchers reported that in 20-week pilot study of lifestyle modification including diet, diet plus aerobic exercise (30 to 45 minute walking and running for five weeks) and diet plus endurance and resistance exercise (30 to 45 minute running on treadmill 3 times a week and endurance exercise two times a week), exercise training significantly changed body composition and reduction in fat mass in comparison with mere diet (Palomba et al., 2008).

Vigorito et al., reported that bicycling for 40 minutes three times a week for 3 months in obese patients

with PCOS led to an improvement in insulin sensitivity (2007). Qiu et al., demonstrated that two weeks swimming in testosterone propionate-induced PCOS rat can improve insulin sensibility, decrease serum androgen levels and recover normal ovarian morphology (2009). Homa et al. indicated voluntary exercise improves estrous cyclicity in prenatally-androgenized female mice (2015).

Diane et al., stated energy-restricted diet combined with or without voluntary exercise (4 h/day) for 8 weeks decreased total body weight gain, body fat mass and fasting plasma triglycerides (2015).

Some other researches demonstrated that training led to an increase in the oxidation of fatty acid, reduced body weight and central fat, improvement in insulin sensitivity and increase in antioxidant which restored ovarian function in PCOS.

Physiological explanation for the effect of regular exercise on the decrease of oxidative stress in PCOS is based on hormesis concept. It is considered as a particular dose- response relationship in which a low dose of a substance such as low concentrations of reactive oxygen is stimulatory to induce expression of antioxidant enzymes and a high dose is inhibitory (Radak et al., 2005; Li et al., 2006)

When the exercise is exhaustive and associated with fatigue due to increase of free radicals and lack of sufficient of antioxidant can be deleterious. However, regular exercise likely causes adaptation in athletes' antioxidant system that can decrease harmful complications from free radicals produced during exercise. Therefore exercise act as double edged sword: when the intensity of exercise is high it causes oxidative stress but when the intensity of exercise is moderate, it increases the expression of antioxidant enzymes. Consistent with research that done in this context it can be concluded that exercise has beneficial effect at improvement of PCOS (Gomez-Cabrera et al., 2008)

Furthermore, in the PCOS exercise group the exercise could mediated sympathetic down-regulation. Prolonged stimulation of muscle afferent such as observed with regular physical exercise, could improve ovarian morphology resembling that observed in this study (Barria et al., 1993)

However, the potential mechanisms for the decreased serum testosterone levels by exercise remain unknown. (Barria et al., 1993; Lara et al., 2002)

Ovarian morphology analysis showed that there were prominent PCOS-like changes with cystic follicles appearance and the accumulation of atretic follicles after administration of estradiol valerat. After eight-week exercise (treadmill and running wheel), general ovarian morphology in PCOS exercise groups were similar to control and indicating a positive effect of exercise training on ovarian morphology.

Ovarian morphology in PCOS exercise groups represented the reduction in numbers of antral and atretic follicles, and increase in corpora lutea numbers. Similar ovarian morphological changes were also found by Mannerås et al. (2009) and Manni et al. (2005) which reported that voluntary exercise prevented the occurrence of polycystic ovary.

## Conclusion

Conclusively, our finding shows for the first time that EV-induced PCOS in rats is associated with an increased TOS and testosterone and decreased TAC. We also demonstrate that voluntary exercise (running wheel exercise) could decrease TOS and testosterone with no effect on TAC. However, treadmill exercise almost normalizes these features (decreased testosterone and TOS and increased TAC). Both of paradigms of exercise normalizes ovarian morphology. It is thus possible that exercise may be a beneficial intervention in the treatment of anovulation and possibly in the prevention of human PCOS.

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## Conflict of interest

There is no conflict of interest in this article.

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