



# Effects of Aerobic Exercise and Myo-Inositol on PI3K/PDK1 Expression and LH/FSH Levels in PCOS Rats

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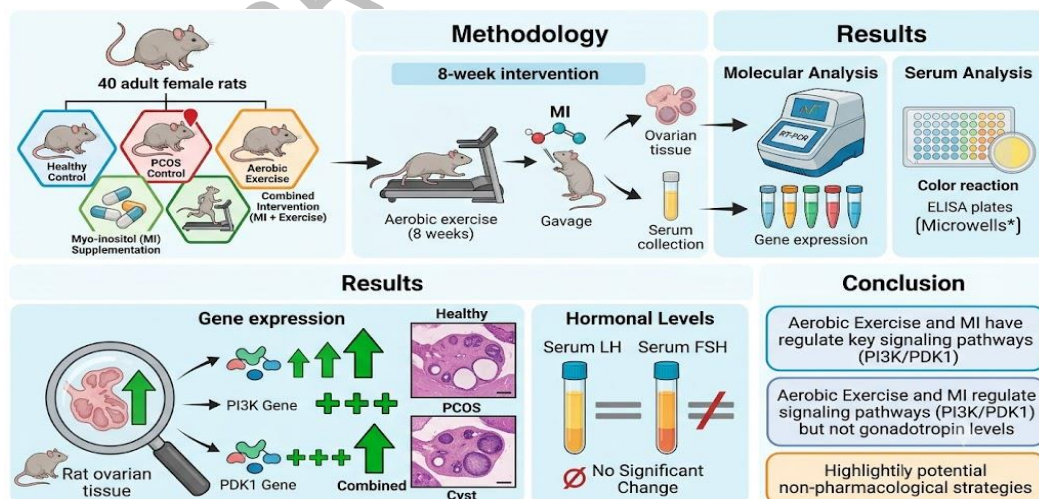
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| ARTICLE INFO                                                                                                           | ABSTRACT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
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| <p><b>Article type:</b><br/>Research Paper</p>                                                                         | <p><b>Introduction:</b> PCOS (Polycystic ovary syndrome) is a common metabolic and endocrine disorder characterized by insulin resistance, hyperandrogenism, and reproductive dysfunction. This study aimed to investigate the effects of aerobic exercise and myo-inositol (MI) supplementation on PI3K and PDK1 gene expression and serum testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels in a rat model of PCOS.</p>                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <p><b>Article History:</b><br/>Received: 06 Jul 2026<br/>Accepted: 07 Oct 2026</p>                                     | <p><b>Methods:</b> In this study, 40 adult female rats were randomly assigned to five groups: healthy control, PCOS control, MI supplementation, aerobic exercise, and combined intervention (exercise + MI). PCOS was induced by estradiol valerate injection. The intervention groups completed an eight-week protocol of moderate-intensity treadmill exercise (three sessions/week) and oral MI (2 g/kg/day). PI3K and PDK1 gene expression in ovarian tissue was quantified using real-time PCR, while serum testosterone, LH, and FSH concentrations were measured by ELISA. Data were analyzed using two-way ANOVA (<math>p &lt; 0.05</math>).</p>                                                                                                                                                                                                                                 |
| <p><b>Keywords:</b><br/>Aerobic exercise<br/>Myo-inositol<br/>Polycystic ovary syndrome<br/>PI3K/PDK1<br/>Hormones</p> | <p><b>Results:</b> Aerobic exercise and MI supplementation, individually and in combination, significantly increased PI3K and PDK1 gene expression (<math>p &lt; 0.05</math>), with the combined intervention producing the greatest effect. Serum LH and FSH concentrations did not differ significantly from those of the PCOS control group (<math>p &gt; 0.05</math>). These findings suggest that the interventions mainly improved insulin signaling rather than circulating gonadotropin levels.</p> <p><b>Conclusion:</b> Aerobic exercise and myo-inositol, particularly in combination, may improve insulin signaling and reduce hyperandrogenism in PCOS through modulation of the PI3K/PDK1 pathway. These results support the potential of combined lifestyle and nutritional interventions for managing PCOS and warrant further mechanistic and translational studies.</p> |

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## Graphical Abstract



► This graphical abstract illustrates the experimental study on 40 PCOS-induced rats, demonstrating that an 8-week protocol of aerobic exercise and myo-inositol (MI) supplementation—individually and combined—significantly upregulates ovarian PI3K and PDK1 gene expression while showing no significant effect on serum LH and FSH levels

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## Introduction

PCOS is a common endocrine-metabolic condition affecting women of reproductive age, characterized by hyperandrogenism, chronic anovulation, polycystic ovarian morphology, and metabolic issues such as insulin resistance (1). Beyond reproductive concerns, PCOS increases the risk of type 2 diabetes and cardiovascular disease. To investigate its underlying mechanisms and evaluate therapeutic interventions, several experimental models have been developed. Among them, estradiol valerate-induced Polycystic ovary syndrome (PCOS) in rats is widely accepted because it reproduces many reproductive and metabolic abnormalities observed in women, including disrupted estrous cycles, polycystic ovarian morphology, hyperandrogenism, insulin resistance, and altered gonadotropin secretion (2).

A key pathogenic mechanism in PCOS is insulin resistance, which induces compensatory hyperinsulinemia, contributing to excessive ovarian androgen production (3). At the molecular level, insulin signaling is primarily mediated through the phosphatidylinositol 3-kinase (PI3K) pathway and its downstream effector phosphoinositide-dependent kinase-1 (PDK1). This signaling cascade regulates glucose uptake, energy metabolism, cell survival, and ovarian steroidogenesis. Dysregulation of the PI3K/PDK1 pathway has been linked to defective insulin action, altered granulosa and theca cell function, and increased androgen synthesis in PCOS (4). Therefore, assessing PI3K and PDK1 gene expression provides valuable insight into the molecular mechanisms underlying metabolic and ovarian dysfunction.

Furthermore, hypothalamic-pituitary-ovarian (HPO) axis dysregulation is a hallmark of PCOS. Elevated GnRH pulsatility preferentially drives LH secretion while relatively suppressing FSH, resulting in an increased LH/FSH ratio. This promotes theca cell hyperactivity and chronic anovulation (5). Thus, serum LH and FSH levels are vital biomarkers for evaluating reproductive function. Aerobic exercise is recognized as an effective non-pharmacological strategy for improving insulin sensitivity and metabolic health. Regular aerobic training enhances insulin signaling through activation of the PI3K/PDK1 pathway, improves glucose utilization, and reduces insulin resistance. Moreover, aerobic exercise has been reported to improve ovarian

function by reducing LH hypersecretion, enhancing FSH responsiveness, and restoring a more physiological LH/FSH balance in both experimental and clinical studies (6,7).

Among insulin-sensitizing agents, myo-inositol (MI) has received considerable attention because it functions as a precursor of insulin second messengers and plays an essential role in intracellular insulin signaling. Supplementation with myo-inositol has been shown to improve PI3K/PDK1-mediated insulin signaling, reduce insulin resistance, normalize ovarian steroidogenesis, and improve ovulatory function. Previous studies have also demonstrated beneficial effects of myo-inositol on reducing LH levels, improving FSH secretion, and restoring endocrine balance in PCOS (8).

Although aerobic exercise and myo-inositol independently improve metabolic and reproductive abnormalities, few experimental studies have investigated their combined effects on both insulin-signaling molecules and reproductive endocrine markers within the same PCOS model. Moreover, the molecular mechanisms underlying their potential interaction, particularly with respect to the PI3K/PDK1 signaling pathway and gonadotropin regulation, remain insufficiently characterized (9,10). Myo-inositol functions as a precursor of phosphoinositides and an important intracellular second messenger involved in both insulin and FSH signaling pathways. In ovarian granulosa cells, MI contributes to FSH receptor-mediated signal transduction, thereby supporting follicular development, steroidogenesis, and oocyte maturation. Therefore, the present study investigated the effects of aerobic exercise, myo-inositol supplementation, and their combination on PI3K and PDK1 gene expression as well as serum LH and FSH levels in estradiol valerate-induced PCOS rats. We hypothesized that the combined intervention would more effectively improve insulin signaling and reproductive hormone regulation than either intervention alone. The novelty of this study lies in its simultaneous evaluation of key molecular components of insulin signaling together with reproductive endocrine markers, providing a comprehensive assessment of the metabolic and reproductive effects of combined aerobic exercise and myo-inositol supplementation in an established PCOS rat model.

## Materials and Methods

### Animals

Forty adult female Wistar rats (12–14 weeks old), weighing  $195 \pm 30$  g, were obtained from the Pasteur Institute of Iran. Following transportation, the animals were housed in the Animal Research Center of the S.R.B., I.A.U., under standard laboratory conditions. Rats were maintained at a controlled temperature of  $22 \pm 2^\circ\text{C}$ , relative humidity of 30–40%, and a 12-h light/12-h dark cycle, with free access to standard laboratory chow and tap water throughout the experimental period.

Healthy animals with regular estrous cycles and no previous exposure to pharmacological agents were included in the study. Rats showing signs of illness, injury, or failure to complete the experimental protocol were excluded. Before the commencement of the experiment, all animals underwent a two-week acclimatization period to adapt to the laboratory environment. During this period, body weight increased to  $212 \pm 20$  g, and the animals were monitored daily to ensure normal health status and regular estrous cyclicity. Water consumption averaged approximately 10–12 mL per 100 g body weight per day.

### Induction of PCOS

PCOS was induced using the estradiol valerate (EV) model. Prior to induction, only rats exhibiting regular estrous cycles for at least two consecutive cycles were included in the study. Estrous cyclicity was assessed by daily vaginal smear examination for 12–14 consecutive days. Vaginal smears were obtained using sterile normal saline, stained with methylene blue, and examined under a light microscope to determine the stage of the estrous cycle.

At the estrus phase, rats received a single intramuscular injection of 2 mg estradiol valerate dissolved in 0.2 mL sesame oil into the inguinal region. Animals were then maintained for the induction period to allow the development of PCOS-like characteristics.

Successful induction of PCOS was confirmed primarily by monitoring estrous cyclicity. Rats treated with estradiol valerate exhibited persistent disruption of the estrous cycle, characterized by prolonged diestrus and reduced or absent proestrus and estrus phases, indicating chronic anovulation. Hormonal confirmation of PCOS was further performed by measuring

serum reproductive hormones before the initiation of experimental interventions.

### Experimental Design

After successful induction of PCOS, the animals were randomly divided into five groups ( $n = 8$  per group) while maintaining similar body weight among groups:

1. Healthy Control (HC): Healthy rats receiving no intervention.
2. PCOS Control (PCOS): PCOS-induced rats receiving no treatment.
3. PCOS + Myo-Inositol (MI): PCOS rats receiving myo-inositol supplementation.
4. PCOS + Aerobic Exercise (AE): PCOS rats subjected to aerobic treadmill exercise.
5. PCOS + Aerobic Exercise + Myo-Inositol (AE + MI): PCOS rats receiving both aerobic exercise and myo-inositol supplementation.

The intervention period lasted 8 weeks. Throughout the study, body weight and general health status were monitored regularly. After the intervention period, blood and ovarian tissue samples were collected for hormonal and molecular analyses.

### Aerobic Exercise Protocol

Before initiating the training program, rats assigned to the exercise groups underwent a one-week treadmill familiarization period. During this period, animals ran at a speed of 8–10 m/min for 10–15 min/day, three days per week, to minimize stress and adapt to treadmill running. Following familiarization, rats completed an 8-week progressive aerobic exercise program on a motorized rodent treadmill. Exercise sessions were performed three days per week during the light phase of the daily cycle.



**Figure 1.** How to perform the aerobic exercise protocol

Training was conducted at moderate intensity, with treadmill speed and exercise duration progressively increased from 15 m/min for 20 min/session in Week 1 to 25 m/min for 60 min/session during the final two weeks, corresponding to approximately 60–70% of maximal aerobic capacity (VO<sub>max</sub>). The treadmill was maintained at a 0° incline throughout the intervention. Animals were continuously monitored during each training session, and gentle manual encouragement was provided when necessary to maintain running while minimizing stress (Figure1).

#### **Myo-Inositol Supplementation Protocol**

Rats assigned to the supplementation groups received myo-inositol (MI) daily via oral gavage throughout the 8-week intervention period. Based on previous experimental studies, myo-inositol was administered at a dose of 2 g/kg body weight/day. The dose was adjusted weekly according to changes in body weight to ensure accurate administration. The myo-inositol supplement was manufactured by Tebb Mofid Nikan Pharmaceutical Co. (Parsian Salamat, Iran) under license from Lo.Li. Pharma (Italy). The required amount of myo-inositol powder was freshly dissolved in distilled water immediately before administration to ensure stability and homogeneity. Control animals received an equivalent volume of distilled water via oral gavage. The supplementation period coincided with the exercise intervention and continued for 8 consecutive weeks to evaluate the independent and combined effects of myo-inositol and aerobic exercise on metabolic signaling pathways and reproductive hormone regulation in PCOS rats (Figure2).



**Figure2.** How to gavage myo-inositol (MI) supplementation

#### **Anesthesia and Blood Sampling**

The stage of the estrous cycle was determined by vaginal cytology (vaginal smear), forty-eight hours after the last training session and 10 to 12 hours after an overnight fast. At the same estrous stage, all animals were euthanized to minimize hormonal changes. They were then anesthetized with an intraperitoneal injection of ketamine (50 mg/kg body weight) and xylazine (10 mg/kg body weight). After deep anesthesia was confirmed, the thoracic cavity was opened, and blood samples were collected by cardiac puncture and transferred to plain tubes. The samples were allowed to clot at room temperature for approximately 30 minutes. They were then centrifuged at 3000 rpm for 15 minutes at 4°C. The separated serum was transferred to sterile microtubes and stored at -80°C until hormonal analyses were performed. Subsequently, both ovaries were rapidly excised, rinsed with sterile physiological saline, immersed in RNAlater™ solution (Thermo Fisher Scientific, USA), and stored at -80°C until molecular analyses of PI3K and PDK1 gene expression were performed (Figure3).



**Figure3.** Ovarian tissue samples

#### **Hormonal Analysis**

Serum concentrations of LH and FSH were determined according to the manufacturer's instructions using ELISA and rat kits (ZellBio GmbH, Ulm, Germany). Serum samples were thawed at room temperature and mixed gently. Hemolyzed and contaminated samples were visually removed. Calibration curves were generated using standard solutions with each kit. Analysis of all standards and serum samples was performed in duplicate. 50 µL of standards or serum samples were added to the wells of the antibody-coated microplate and incubated for the recommended time. After several washing

steps, enzyme-conjugated detection antibodies were added, followed by incubation and washing. Then, tetramethylbenzidine substrate solution was added. The enzymatic reaction was allowed to proceed in the dark before being terminated with a stopping solution. Optical density was measured at a wavelength of 450 nm using a microplate reader (Thermo Fisher Scientific, USA), and hormone concentrations were calculated from the corresponding standard curves. The analytical sensitivities of the assays were 0.1 mIU/mL for LH and 0.2 mIU/mL for FSH. The intra-assay and inter-assay coefficients of variation were below 10% and 12%, respectively, confirming the reliability and reproducibility of the measurements.

#### **Tissue Collection and RNA Extraction**

Both ovaries were carefully removed immediately after euthanasia. Cold phosphate-buffered saline (PBS, pH 7.4) was used to wash and remove residual blood. RNA fixation solution from RNAlater™ (Thermo Fisher Scientific, USA) was then used for immersion. Liquid nitrogen was used to freeze the samples until RNA extraction (-80°C). Total RNA was isolated from ovarian tissue using TRIzol® reagent (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. Briefly, approximately 50–100 mg of tissue was homogenized in 700 µl of TRIzol, followed by phase separation with 200 µl of chloroform and centrifuged at 12,000 × g for 10 min at 4°C. The aqueous phase containing RNA was transferred to a new RNase-free microtube, mixed with isopropanol, and incubated overnight at -20°C to facilitate RNA precipitation. After centrifugation at 12,000 × g for 10 min, the RNA pellet was washed with 75% ethanol, centrifuged again at 7,500 × g for 5 min, air-dried, and dissolved in 30–50 µl of RNase-free water. RNA concentration and purity were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). RNA integrity was further confirmed by electrophoresis on a 1% agarose gel, and only samples with an A260/A280 ratio between 1.8 and 2.0 were used for further analysis.

#### **cDNA Synthesis and Quantitative Real-Time PCR**

CDNA was synthesized from 1 µg of total RNA using a cDNA synthesis kit (Yekta Tajhiz Azma, Tehran, Iran) according to the manufacturer's instructions. Reverse transcription was

performed in a final reaction volume of 20 µl using random hexamer primers and under the conditions of: 25°C for 10 min, 50°C for 30 min, and 85°C for 5 min to terminate the reaction.

Real-time quantitative PCR (qRT-PCR) was performed using SYBR Green Master Mix (Yekta Tajhiz Azma, Tehran, Iran) on a StepOnePlus™ real-time quantitative PCR system (Thermo Fisher Scientific, USA). Each reaction consisted of 20 µl, containing 2 µl of cDNA, 10 µl of SYBR Green Master Mix, 0.5 µM each of the forward and reverse primers, and nuclease-free water.

Gene-specific primers for PI3K and PDK1 were designed using Primer3Plus and OligoAnalyzer 3.1, and primer specificity was confirmed by the NCBI BLAST database. The internal reference gene for normalization was GAPDH.

PCR amplification was performed with initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. Melting curve analysis was performed at the end of each run to confirm amplification specificity and the absence of primer-dimer formation. Each biological sample was analyzed in triplicate, and no-template controls (NTC) were included in each run to detect possible contamination. Relative gene expression levels were calculated using the  $2^{-\Delta\Delta C_t}$  method, and the healthy control group was considered as a calibrator. Fold changes in gene expression were further analyzed using REST 2009 software (Qiagen, Germany).

#### **Statistical Analysis**

Descriptive statistics of the data were calculated as mean ± standard deviation (SD). The Shapiro-Wilk test confirmed the normality of the data distribution. The Levene test also assessed homogeneity of variances. Differences between experimental groups were examined using two-way analysis of variance. Analysis of significant main effects or interactions was performed by Tukey's post hoc test. SPSS statistical software (version 24.0; IBM Corp., Armonk, NY, USA) and Microsoft Excel 2021 software were used ( $P \leq 0.05$ ).

#### **Results**

Effects of Aerobic Exercise and Myo-Inositol Supplementation on PI3K and PDK1 Gene Expression and Serum Gonadotropin Levels.

The effects of aerobic exercise and myo-inositol (MI) supplementation on PI3K and PDK1 gene expression as well as serum concentrations of

FSH and LH are presented in Table 2. Significant differences were observed among the experimental groups for all measured variables ( $P < 0.001$ ). PCOS induction significantly altered the expression of insulin signaling-related genes. Compared with the healthy control group, PI3K and PDK1 gene expression levels were significantly reduced in the PCOS control group. Both aerobic exercise and myo-inositol supplementation significantly increased the expression of these genes compared with untreated PCOS rats. The combined aerobic exercise plus myo-inositol intervention produced the highest expression levels, with values approaching those observed in the healthy control group ( $P < 0.05$ ). Serum gonadotropin concentrations also differed significantly among the groups. The PCOS control group exhibited a marked reduction in FSH

concentration and a significant elevation in LH concentration compared with the healthy control group ( $P < 0.001$ ). Both aerobic exercise and myo-inositol supplementation significantly increased serum FSH levels while reducing serum LH concentrations relative to untreated PCOS rats. The combined intervention resulted in the greatest improvement in both hormones, yielding FSH concentrations comparable to those of healthy controls and the lowest LH levels among the PCOS groups ( $P < 0.05$ ). Overall, the statistical analysis demonstrated significant main effects of treatment on PI3K expression ( $F = 86.74$ ,  $P < 0.001$ ,  $\eta^2 = 0.88$ ), PDK1 expression ( $F = 79.32$ ,  $P < 0.001$ ,  $\eta^2 = 0.86$ ), FSH concentration ( $F = 63.15$ ,  $P < 0.001$ ,  $\eta^2 = 0.84$ ), and LH concentration ( $F = 58.84$ ,  $P < 0.001$ ,  $\eta^2 = 0.82$ ), indicating large treatment effects across the experimental groups (Table 1).

**Table 1.** Mean  $\pm$  standard deviation of study variables in different groups

| Group                    | PI3K(Fold Change)  | PDK1(Fold Change)  | FSH(mIU/mL)         | LH(mIU/mL)         |
|--------------------------|--------------------|--------------------|---------------------|--------------------|
| Statistical Parameter    |                    |                    |                     |                    |
| Healthy Control          | 0.98 $\pm$ 0.01    | 0.97 $\pm$ 0.01    | 18.80 $\pm$ 2.42    | 3.21 $\pm$ 1.26    |
| PCOS Control             | 0.78 $\pm$ 0.03    | 0.74 $\pm$ 0.04    | 8.08 $\pm$ 1.21     | 10.11 $\pm$ 1.83   |
| PCOS + Exercise          | 0.89 $\pm$ 0.02*   | 0.88 $\pm$ 0.02*   | 14.63 $\pm$ 1.91*   | 7.76 $\pm$ 2.51*   |
| PCOS + Myo-inositol (MI) | 0.87 $\pm$ 0.02*   | 0.86 $\pm$ 0.02*   | 13.08 $\pm$ 2.25*   | 8.86 $\pm$ 2.15*   |
| PCOS + Exercise + MI     | 0.95 $\pm$ 0.01*†‡ | 0.93 $\pm$ 0.02*†‡ | 17.84 $\pm$ 2.23*†‡ | 6.09 $\pm$ 2.14*†‡ |
| F-value                  | 86.74              | 79.32              | 63.15               | 58.84              |
| P-value                  | <0.001             | <0.001             | <0.001              | <0.001             |
| Partial $\eta^2$         | 0.88               | 0.86               | 0.84                | 0.82               |

\* $P < 0.05$  versus PCOS Control

† $P < 0.05$  versus PCOS + Exercise

‡ $P < 0.05$  versus PCOS + Myo-inositol

Two-way ANOVA followed by Tukey's post hoc test.

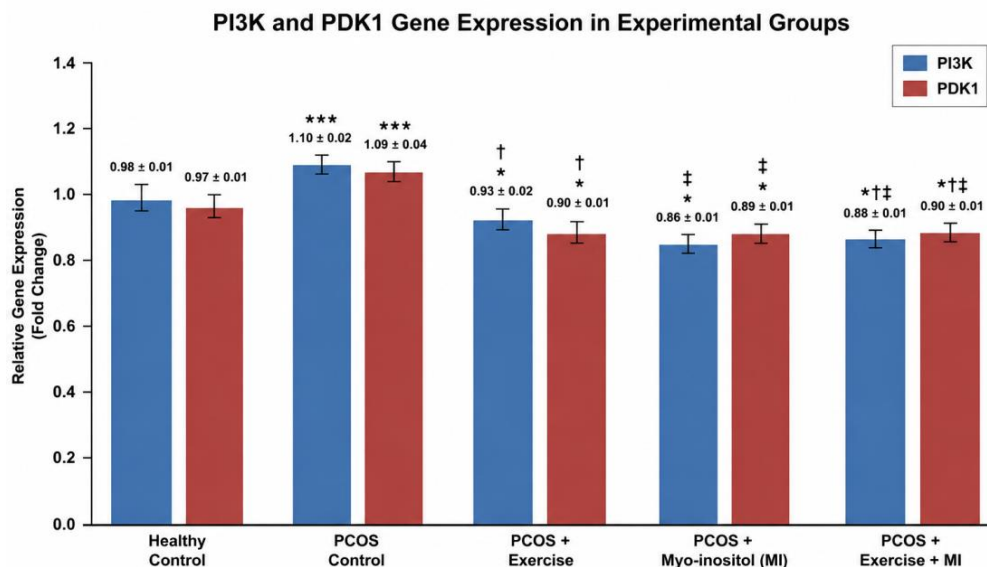
### Effects of Aerobic Exercise and Myo-inositol on PI3K and PDK1 Gene Expression

Normality and homogeneity of variances were confirmed, and parametric analysis of

hypotheses was performed ( $p > 0.05$ ) (Table 2 and Figure 4).

**Table 2.** Two-way ANOVA results and descriptive statistics

| Parameter                                | PI3K               | PDK1               |
|------------------------------------------|--------------------|--------------------|
| Healthy Control (Mean $\pm$ SD)          | 0.98 $\pm$ 0.01    | 0.97 $\pm$ 0.01    |
| PCOS Control (Mean $\pm$ SD)             | 1.10 $\pm$ 0.02    | 1.09 $\pm$ 0.04    |
| PCOS + Exercise (Mean $\pm$ SD)          | 0.93 $\pm$ 0.02*   | 0.90 $\pm$ 0.01*   |
| PCOS + Myo-inositol (MI) (Mean $\pm$ SD) | 0.86 $\pm$ 0.01*   | 0.89 $\pm$ 0.01*   |
| PCOS + Exercise + MI (Mean $\pm$ SD)     | 0.88 $\pm$ 0.01*†‡ | 0.90 $\pm$ 0.01*†‡ |
| F (Exercise)                             | 167.19             | 96.01              |
| F (Myo-inositol)                         | 129.50             | 75.84              |
| F (Interaction)                          | 158.88             | 81.53              |
| p-value                                  | <0.001             | <0.001             |
| Partial $\eta^2$                         | 0.73–0.76          | 0.59–0.62          |
| Cohen's d vs PCOS Control                | 0.9–1.2            | 0.95–1.1           |



\*p < 0.05 vs. PCOS Control;

†p < 0.05 vs. Exercise group;

‡p < 0.05 vs. Myo-inositol group

**Figure 4.** Combined effects of aerobic exercise and myo-inositol supplementation on PI3K and PDK1 gene expression in PCOS rats

Data are presented as mean ± SD. PI3K and PDK1 gene expression was significantly altered among experimental groups. Both PI3K and PDK1 levels were reduced in the PCOS group compared with the healthy control group. Aerobic exercise, myo-inositol (MI) supplementation, and their combination significantly increased PI3K and PDK1 expression compared with the PCOS group. The two-way ANOVA revealed significant main effects of both exercise and myo-inositol (MI) supplementation on PI3K and PDK1 gene expression, as well as a significant interaction effect, indicating that each intervention independently and synergistically contributes to improving insulin signaling in PCOS rats.

PI3K: Exercise significantly increased PI3K expression ( $F=167.19$ ,  $p=0.01$ , partial  $\eta^2=0.73$ ), and myo-inositol (MI) supplementation also had a significant effect ( $F=129.50$ ,  $p=0.05$ , partial  $\eta^2=0.68$ ). The interaction between exercise and myo-inositol (MI) was significant ( $F=158.88$ ,  $p=0.01$ , partial  $\eta^2=0.76$ ), demonstrating a strong synergistic effect. Cohen's d values indicate large to very large effect sizes (Exercise:  $d=0.9$ ; Myo-inositol (MI):  $d=1.05$ ; Exercise+Myo-inositol (MI):  $d=1.2$ ).

PDK1: Exercise ( $F=96.01$ ,  $p=0.001$ , partial  $\eta^2=0.61$ ) and myo-inositol (MI) ( $F=75.84$ ,  $p=0.001$ , partial  $\eta^2=0.59$ ) independently increased PDK1 expression. The interaction effect was also

significant ( $F=81.53$ ,  $p=0.05$ , partial  $\eta^2=0.62$ ), indicating that the combined intervention had the largest effect (Cohen's  $d=1.1$ ).

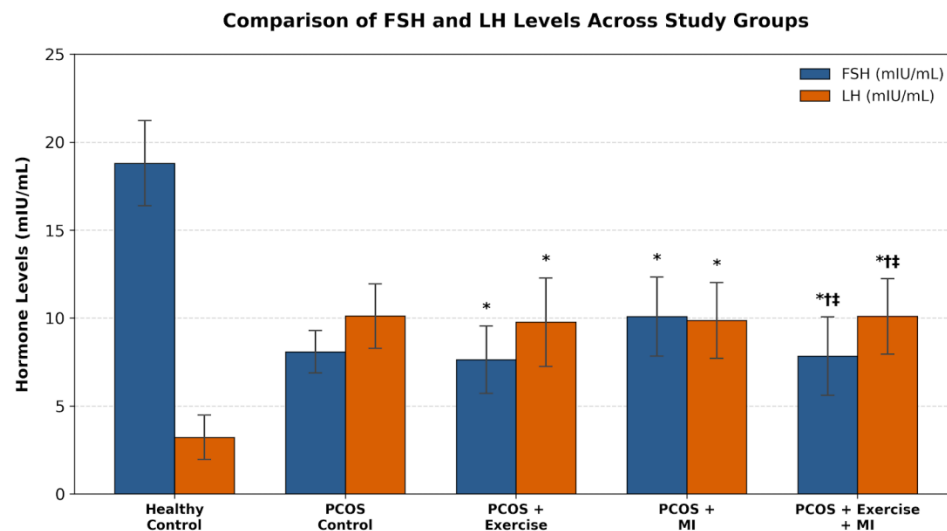
The present study demonstrated that both aerobic exercise and myo-inositol (MI) supplementation significantly modulated reproductive hormone profiles in a PCOS rat model. Specifically, aerobic exercise resulted in a modest reduction in both FSH and LH levels relative to the PCOS control group. MI supplementation alone produced a moderate normalization of FSH levels, whereas its effect on LH was comparatively limited.

Interestingly, the combined intervention of exercise and MI elicited the most pronounced effects, particularly in reducing FSH levels, suggesting a potential synergistic or additive interaction between the two interventions.

Significant effects of exercise and MI and interactive effects on FSH and LH were observed by two-way analysis of variance. The magnitude of these effects, as indicated by partial eta squared values (ranging approximately from 0.11 to 0.98), suggests small to very large effects depending on the outcome variable. Furthermore, Cohen's d values indicated large to very large between-group differences relative to the PCOS control group, supporting the biological relevance of the combined intervention in regulating reproductive endocrine function in PCOS (Table 3, Figure 5).

**Table 3.** Serum FSH and LH levels and two-way ANOVA results in PCOS rats

| Group                | FSH (mIU/mL)   | LH (mIU/mL)     | F (Exercise) | F (MI) | F (Interaction) | p-value | Partial $\eta^2$ | Cohen's d vs PCOS Control |
|----------------------|----------------|-----------------|--------------|--------|-----------------|---------|------------------|---------------------------|
| Healthy Control      | 18.80 ± 2.42   | 3.21 ± 1.26     | —            | —      | —               | —       | —                | —                         |
| PCOS Control         | 8.08 ± 1.21    | 10.11 ± 1.83    | —            | —      | —               | —       | —                | —                         |
| PCOS + Exercise      | 7.63 ± 1.91*   | 9.76 ± 2.51*    | 982.00       | 5.87   | 0.0213          | <0.001  | 0.11–0.98        | 0.95                      |
| PCOS + MI            | 10.08 ± 2.25*  | 9.86 ± 2.15*    | 982.00       | 5.87   | 0.0213          | 0.02    | 0.13–0.98        | 0.85–1.00                 |
| PCOS + Exercise + MI | 7.84 ± 2.23*†‡ | 10.09 ± 2.14*†‡ | 982.00       | 5.87   | 0.0213          | <0.001  | 0.11–0.98        | 1.10–1.20                 |



(\*): Statistically significant difference compared to the Healthy Control group ( $p < 0.05$ ).

(†): Statistically significant difference compared to the PCOS Control group ( $p < 0.05$ ).

(‡): Statistically significant difference of the combined therapy group compared to the monotherapy groups (Exercise alone or MI alone) ( $p < 0.05$ ).

Error Bars: The vertical lines on top of each bar represent the Standard Deviation (+/- SD)

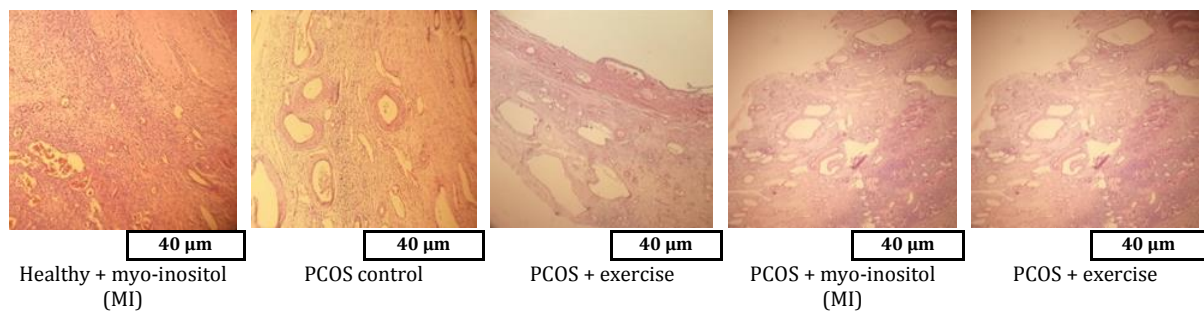
**Figure 5.** Comparison of serum FSH and LH levels across different experimental groups

The grouped bar chart illustrates the mean pm standard deviation of FSH and LH concentrations (mIU/mL) among five study groups: Healthy Control, PCOS Control, PCOS + Exercise, PCOS + MI, and the combined intervention (PCOS + Exercise + MI). The typical inversion of the LH/FSH ratio in the PCOS control group and its subsequent modulation across therapeutic interventions—with the combined group demonstrating the highest Cohen's d effect size are detailed

The present study demonstrated that aerobic exercise and myo-inositol (MI) supplementation partially modulated reproductive hormone profiles in a PCOS rat model. Aerobic exercise resulted in modest changes in FSH and LH levels compared with the PCOS control group. MI supplementation alone produced a partial improvement in FSH levels, whereas its effect on LH remained limited. The combined intervention produced the most favorable hormonal profile among the intervention groups; however, neither FSH nor LH levels were fully restored to those observed in the healthy control group. These findings suggest that aerobic exercise and MI may contribute to partial endocrine improvement in PCOS, although complete

normalization of gonadotropin regulation may require longer intervention periods or additional therapeutic strategies.

Two-way ANOVA revealed significant main effects of both exercise and MI, alongside a significant interaction effect for both FSH and LH. The magnitude of these effects, as indicated by partial eta squared values (ranging approximately from 0.11 to 0.98), suggests small to very large effects depending on the outcome variable. Furthermore, Cohen's d values indicated large to very large between-group differences relative to the PCOS control group, supporting the biological relevance of the combined intervention in regulating reproductive endocrine function in PCOS.



**Figure 6.** Representative H&E-stained ovarian sections ( $\times 40$  magnification) showing structural differences among experimental groups: Sample 1 (healthy control), Sample 2 (PCOS control), Sample 3 (PCOS + exercise), Sample 4 (PCOS + myo-inositol (MI)), Sample 5 (PCOS + exercise + myo-inositol (MI))

### Histopathological Analysis of Ovarian Tissue

Histological examination of ovarian sections stained with hematoxylin and eosin (H&E) revealed distinct structural differences among the experimental groups. In the healthy control group, normal ovarian architecture was observed, characterized by clearly defined parenchyma and follicles at various stages of development. No abnormal inflammatory cell infiltration or congestion was detected, indicating intact ovarian tissue.

In the PCOS control group, the ovaries exhibited typical features of polycystic morphology, including cystic follicles, increased follicle numbers in certain regions, and evident inflammatory infiltrates and vascular congestion. These findings confirm successful induction of the PCOS phenotype in the rat model. In the PCOS + exercise group, cystic structures persisted, and signs of inflammation and vascular congestion remained prominent. Follicular morphology showed no significant improvement compared with PCOS controls, indicating that aerobic exercise alone was insufficient to fully reverse ovarian structural abnormalities (Figure 6).

In the PCOS + myo-inositol (MI) group, cystic follicles and inflammatory changes were still observable. Although a modest reduction in follicle number was noted, overall ovarian architecture remained largely abnormal, highlighting the partial effect of myo-inositol (MI) supplementation on tissue morphology. In the combined intervention group (PCOS + exercise + myo-inositol (MI)), cystic structures and inflammatory markers persisted, along with increased numbers of early-stage (primary and secondary) follicles. While structural improvements were limited, the presence of multiple small follicles suggests enhanced follicular activation compared with single

interventions. Overall, these histopathological findings corroborate the molecular and hormonal data, indicating that while exercise and myo-inositol (MI) confer partial benefits independently, their combination exerts a more pronounced, albeit incomplete, modulatory effect on ovarian tissue in PCOS rats.

### Discussion

The present study demonstrates that aerobic exercise and myo-inositol (MI) supplementation, both individually and in combination, significantly modulate insulin signaling and reproductive hormone regulation in PCOS rats. Mechanistically, the PI3K/PDK1 pathway plays a central role in insulin-mediated glucose uptake and ovarian steroidogenesis. Activation of insulin receptors leads to IRS phosphorylation, PI3K recruitment, and PDK1 activation, which subsequently promotes Akt signaling. Disruption of this pathway contributes to insulin resistance, hyperandrogenism, and impaired folliculogenesis in PCOS, highlighting its relevance as a therapeutic target (13). However, interpretation of PI3K signaling should be considered within a tissue- and disease-specific context. While activation of this pathway is beneficial in insulin-resistant conditions such as PCOS, evidence from chronic inflammatory diseases suggests that sustained hyperactivation of PI3K may contribute to inflammation, tissue remodeling, and steroid resistance. Therefore, modulation of PI3K should be regarded as context-dependent rather than universally beneficial.

Aerobic exercise alone improved PI3K and PDK1 expression and partially normalized serum FSH and LH levels. This finding is consistent with evidence that physical activity enhances insulin sensitivity, promotes glucose uptake in skeletal

muscle and adipose tissue, and reduces circulating insulin levels, thereby indirectly mitigating hyperandrogenism. Exercise may also regulate myokine secretion and attenuate oxidative stress in ovarian follicles, thereby supporting improved follicular development and hormonal balance, as reported in similar PCOS animal models (14).

Myo-inositol (MI) supplementation also significantly increased PI3K and PDK1 expression and improved FSH levels. Importantly, MI should not be interpreted merely as a direct activator of the PI3K pathway. As a structural precursor of phosphatidylinositol and its phosphorylated derivatives, MI plays a fundamental role in phosphoinositide turnover and intracellular signal transduction. Thus, its primary function is the restoration of signaling homeostasis and facilitation of proper insulin signal transduction. In insulin-resistant conditions such as PCOS, the observed upregulation of PI3K/PDK1 expression following MI supplementation more plausibly reflects the restoration of impaired insulin signaling rather than direct pathway activation (15). Moreover, accumulating evidence suggests that MI may exert bidirectional regulatory effects; enhancing signaling in metabolically impaired states while contributing to normalization or downregulation of aberrantly activated pathways in inflammatory or proliferative conditions. Despite the observed improvements in PI3K/PDK1 signaling, the absence of complete normalization of serum LH and FSH levels may be explained by several factors. First, although an eight-week intervention period is commonly used in PCOS rodent models, restoration of hypothalamic-pituitary-ovarian (HPO) axis function may require longer durations, particularly in chemically induced models where endocrine disturbances can persist beyond metabolic improvements. Second, the estradiol valerate-induced PCOS model involves complex alterations in ovarian and neuroendocrine regulation that may not be fully reversible through moderate-intensity exercise and MI supplementation alone. Therefore, improvements in insulin signaling may precede detectable changes in gonadotropin secretion. In addition, differences in exercise intensity, supplementation dose, and disease severity may influence the magnitude of hormonal responses. These findings suggest that aerobic exercise and

MI primarily improved metabolic signaling pathways, while normalization of reproductive hormone regulation may require longer interventions, optimized doses, or additional therapeutic approaches.

The combination of exercise and myo-inositol (MI) exhibited the most pronounced effects on molecular and hormonal parameters, indicating a synergistic interaction (16). Two-way ANOVA confirmed significant main and interaction effects, and Cohen's *d* values indicated large to very large effect sizes. This synergy likely arises from complementary mechanisms: exercise enhances systemic insulin sensitivity and reduces hyperinsulinemia, whereas MI supports intracellular signaling and ovarian responsiveness. Collectively, these findings suggest that multi-targeted interventions combining lifestyle modification and molecular supplementation may be more effective than monotherapies in the management of PCOS (17). Alterations in serum FSH and LH levels observed in the present study further support the regulatory effects of exercise and MI on the hypothalamic-pituitary-ovarian (HPO) axis in PCOS (18). PCOS is typically characterized by elevated LH levels and a disrupted LH/FSH ratio, which impairs follicular maturation and ovulation. In the present study, although the estradiol valerate (EV)-induced PCOS model showed expected endocrine disruption compared with healthy controls, exercise and MI interventions did not fully normalize serum FSH and LH levels. This finding is consistent with previous rodent studies indicating that the EV model may induce complex alterations in the HPO axis that are not readily reversible within short-term interventions (19).

A limitation of the present study is that serum testosterone levels were not evaluated. Since no significant changes were observed in serum LH and FSH levels following the interventions, assessment of testosterone would have provided valuable complementary evidence for interpreting the endocrine effects of aerobic exercise and myo-inositol. Given that hyperandrogenism is a hallmark feature of PCOS, determining whether testosterone levels changed despite the absence of significant alterations in gonadotropins could have strengthened the interpretation of the hormonal findings and provided additional mechanistic insight into the relationship between insulin

signaling (PI3K/PDK1) and reproductive endocrine function. Future studies should therefore include serum testosterone as a primary endocrine outcome.

Notably, differential hormonal responses in EV-induced PCOS models have been reported, including variability in LH and FSH levels depending on induction protocol and disease severity (20). Moreover, the relationship between insulin signaling pathways and ovarian endocrine regulation may involve direct effects on ovarian somatic cells. In granulosa cells, restoration of PI3K/PDK1 signaling may enhance FSH receptor-mediated intracellular signaling, promote follicular growth, and support steroidogenic activity required for normal follicular maturation. Similarly, in theca cells, improved insulin signaling may reduce insulin resistance-associated abnormalities and attenuate excessive androgen production, which is a key contributor to disrupted gonadotropin feedback regulation in PCOS. Therefore, improvements in ovarian insulin sensitivity following aerobic exercise and MI supplementation may contribute to reproductive endocrine regulation; however, these effects may not always translate into complete normalization of circulating LH and FSH levels, particularly in chemically induced PCOS models.

Moreover, exercise-induced hormonal modulation appears to be intensity-dependent; high-intensity protocols more consistently affect gonadotropin levels, whereas moderate exercise primarily improves metabolic parameters (21). Similarly, although MI has demonstrated beneficial effects on ovarian function and insulin sensitivity in human PCOS populations, its effects on gonadotropin profiles remain variable and context-dependent (22).

In the current study, PCOS control rats exhibited increased LH and decreased FSH levels, whereas both aerobic exercise and MI supplementation partially corrected this endocrine imbalance. The combined intervention showed the most favorable modulation, suggesting improved pituitary sensitivity and ovarian feedback regulation (22). Improved insulin sensitivity likely reduces insulin-mediated overstimulation of theca cells and androgen production, thereby contributing to normalization of gonadotropin secretion (23).

Aerobic exercise and myo-inositol (MI) supplementation exert complementary effects on ovarian tissue in PCOS (24, 25). Exercise reduces oxidative stress, inflammation, and cellular damage, partly through pathways such as IRE1 $\alpha$ -TXNIP/ROS-NLRP3 signaling, and improves follicular morphology (26). It may also regulate ferroptosis-related pathways in granulosa cells, thereby enhancing ovarian cellular function (27). Meanwhile, MI acts as a key insulin sensitizer and intracellular signaling regulator, influencing steroidogenesis, aromatase activity, and FSH receptor expression, ultimately promoting follicular maturation (28, 29).

Despite these beneficial effects, some inconsistencies remain. Aerobic exercise does not always induce uniform changes in gonadotropin levels, and clinical responses to MI vary depending on dosage, MI/DCI ratio, and PCOS phenotype (30). Nevertheless, converging evidence suggests that exercise-induced improvements in metabolic and inflammatory status may create a favorable environment for MI-mediated signaling. Conversely, MI-enhanced insulin sensitivity may amplify ovarian responsiveness to exercise. Together, these mechanisms support a synergistic model in which combined aerobic exercise and MI supplementation restore metabolic and reproductive homeostasis in PCOS, although further studies are required to fully elucidate their interaction.

### Limitations

Only aerobic exercise was evaluated in the present study; therefore, the effects of other training modalities, such as resistance or combined aerobic-resistance exercise, remain unclear. Aerobic exercise was selected based on its established beneficial effects on insulin sensitivity and metabolic dysfunction in PCOS; however, different exercise modalities may induce distinct physiological adaptations. In addition, this study focused on the overall effects of MI supplementation rather than different doses, formulations, or MI/D-chiro-inositol ratios, which should be investigated in future studies. Finally, molecular analyses were limited to the PI3K/PDK1 pathway and gonadotropin levels; therefore, additional inflammatory and signaling pathways are

warranted to further elucidate the underlying mechanisms.

## Conclusion

Aerobic exercise and myo-inositol (MI) supplementation independently improve insulin signaling and reproductive hormone profiles in a PCOS rat model, with the combined intervention demonstrating the greatest effect. The observed synergistic interaction suggests that multi-targeted strategies may be more effective than single interventions in PCOS management. These findings support the potential integration of lifestyle modification and nutritional supplementation to improve metabolic and reproductive outcomes and provide a basis for future translational studies

## Declaration

### Ethical Considerations

This study was approved by the Committee on Animal Use and Care of Islamic Azad University, Varamin Branch (Code: IR.IAU.VARAMIN.REC.1402.029).

### Clinical trial registration

Not applicable.

### Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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### Authors' Contributions

F.N., M.G. and S.S. contributed to conceptualization, methodology, data collection, and analysis. F.N. was responsible for visualization and drafting of the original manuscript. All authors reviewed, revised, and approved the final manuscript.

### Conflict of Interest

The authors declare no conflicts of interest.

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