

Effects of different durations of high-intensity interval training or continuous training with/without metformin on lipid profile and malondialdehyde level in a rat model of polycystic ovary syndrome

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Article Info	Abstract
Article history: Received: 29 January 2025 Accepted: 05 February 2025 Available online: 15 February 2026	<p>Polycystic ovary syndrome (PCOS) is a complex endocrine disorder. Exercise is one of the interventions that leads to PCOS improvement. This study aimed to investigate the potential of different durations of high-intensity interval training (HIIT) or continuous training (CT) with/without metformin (MET) in a rat model of PCOS. For this purpose, the rats were randomly placed in seven groups, including control, PCOS, PCOS + CT, PCOS + HIIT, PCOS + MET, PCOS + MET + CT, and PCOS + MET + HIIT. The PCOS was induced by a single intra-muscular injection of 2.00 mg kg⁻¹ estradiol valerate. The concentration of lipid indices showed that serum cholesterol and low-density lipoprotein decreased, and high-density lipoprotein increased with 8 and 12 weeks of exercise training in PCOS + CT, PCOS + HIIT, PCOS + MET + CT, and PCOS + MET + HIIT groups compared to the PCOS group, but no significant difference was found between groups. Triglyceride level decreased in treated groups with 12 weeks of exercise compared to the PCOS group, and there was no significant difference between the treated groups. Finally, serum malondialdehyde level decreased in groups received exercise intervention alone or combined with MET compared to the PCOS group. These findings revealed that HIIT and CT with longer periods with/without MET can lead to improvement of lipid profile and malondialdehyde level of PCOS patients.</p>
Keywords: Continuous training High-intensity interval training Lipid profile Malondialdehyde Polycystic ovary syndrome	

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Introduction

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder. It is a common disorder (6.00 - 21.00%) among women of reproductive age,¹ which is the most prevalent endocrine-metabolic disorder in this group.² According to Rotterdam criteria, PCOS is defined when at least two of the following three features are present: Clinical or biochemical hyperandrogenism, oligo-ovulation or anovulation with menstrual irregularities, and polycystic ovarian morphology.³ Patients with PCOS exhibit adverse reproductive characteristics, cardiovascular risk factors, dyslipidemia, type 2 diabetes, and psychological disorders, such as depression and anxiety.⁴ The economic burden associated with PCOS healthcare due to the pregnancy complications and long-term consequences is enormous.⁵ Therefore, it is essential to provide strategies for the prevention and treatment of this disease.

As mentioned, PCOS patients often develop dyslipidemia.⁴ In the pathophysiology of PCOS dyslipidemia, insulin resistance and hyperandrogenism increase lipolysis induced by catecholamines and release of circulating fatty acids. Finally, free fatty acid flow to the liver stimulates the accumulation and release of very-low-density lipoprotein (VLDL) and increases blood triglyceride (TG) levels.⁶ Additionally, malondialdehyde (MDA) is one of the important end products of lipid peroxidation; therefore, it is one of the most common biomarkers used to assess oxidant status.⁷ Insulin resistance, hyperandrogenism, dyslipidemia, and PCOS-related obesity probably increase MDA levels and also reduce anti-oxidant enzyme levels.⁸ Studies showed that higher oxygen free radical production, evidenced by increased MDA, supports oxidative stress in PCOS.⁹ Additionally, women with PCOS often experience an increase in body weight.^{10,11} This body weight gain may be due to the accumulation of adipose tissue.¹⁰

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Exercise is recognized as a first-line of treatment for PCOS.¹² Exercise can contribute to improving PCOS patients' conditions.¹³ Exercise is a wide word including any pre-planned and structured physical activity.¹⁴ Interval and continuous exercises are types of exercises. Interval training is an intermittent period of effort interspersed by recovery periods, and continuous training (CT) is a continuous effort performed at a constant speed.¹⁵ Exercise can significantly reduce total cholesterol, low-density lipoprotein (LDL), and TG levels while increasing high-density lipoprotein (HDL) level.¹⁶ Rabeiro *et al.* showed a significant decrease in TG and LDL levels after 16 weeks of continuous and interval exercises, while cholesterol and HDL did not show significant changes.¹⁷ Almenning *et al.* also observed that high-intensity CT increased HDL levels.¹⁸ Furthermore, an 8-week combined exercise training (aerobic with resistance) resulted in increase in HDL, decrease in LDL and cholesterol, and no change in TGs levels.¹⁹ Also, it has been shown that exercise training improves oxidative stress in PCOS.²⁰ For example, 12 weeks of aerobic exercise in women with PCOS increased superoxide dismutase levels in line with total anti-oxidant capacity, while MDA levels were reduced.²¹

Metformin (MET) is also a drug used in PCOS to improve the lipid profile.^{22,23} It was found that a 6-month treatment with MET significantly increased HDL levels in obese and non-obese women with PCOS, while LDL and TG levels remained unchanged in both groups.²⁴ There is evidence that combining MET with exercise can offer additional advantages for exercise in PCOS patients.²⁵ Investigations showed that so far no study has been conducted to evaluate the effect of different durations and types of exercise with/without MET on lipid profile and MDA level in PCOS. Therefore, the effects of different durations of high-intensity interval training (HIIT) or CT with/without MET on lipid profile and MDA level in a rat model of PCOS were investigated in this study.

Materials and Methods

Animals. Female Wistar rats (n = 105; 6-week old and average weight of 189.94 ± 26.54 g) were housed under standard laboratory conditions (22.00 °C and 12 hr light/12 hr dark cycle). Rats had free access to food and water. The study was conducted at the Central Laboratory of Urmia University, Urmia, Iran. All experimental procedures of current research were performed based on the mentioned criteria in the Guide for the Care and Use of Laboratory Animals,²⁶ and approved by the Ethical Committee of Research on Laboratory Animals of Urmia University, Urmia, Iran (IR-UU-AEC-3/2).

Assessment of estrous cycle. To establish the regularity of sexual cycle in rats, vaginal smear tests were utilized. Vaginal smears for all rats were conducted daily at 9:00 a.m. To examine vaginal cells, the uterine cervix was

positioned in the suitable position, and vaginal smears were obtained using a swab. Subsequently, each sample was placed on a glass slide, air-dried, fixed with 96.00% alcohol, stained with Hematoxylin and Eosin, and then analyzed using a light microscope (Olympus, Tokyo, Japan). The different stages of rat estrous cycle are characterized by the ratio of epithelial cells, leukocytes, and cornified cells. The pro-estrous stage was characterized by primarily nucleated epithelial cells, estrous stage by the abundance of anuclear cornified cells, metestrous stage by leukocytes and cornified and nucleated epithelial cells, and diestrous stage by a large number of leukocytes and nucleated epithelial cells.²⁷ Finally, animals that had three normal estrous cycles were selected.

Polycystic ovary syndrome induction. The rats were randomly assigned to seven groups (n = 15), including control, PCOS, PCOS + CT, PCOS + HIIT, PCOS + MET, PCOS + MET + CT, and PCOS + MET + HIIT. In PCOS groups, it was induced by a single intra-muscular injection of 2.00 mg kg⁻¹ body weight of estradiol valerate (Aburaihan Pharmaceutical Co., Tehran, Iran), dissolved in 0.20 mL olive oil.^{28,29} The control group received only olive oil injection.³⁰ After 60 days, to ensure induction of PCOS, vaginal smear tests were repeated.³¹ Rats were selected as PCOS, which were in the diestrus phase in three consecutive vaginal smear tests. In the MET groups, a daily dose of 300 mg of MET *per* kg of body weight, dissolved in 2.00 mL of distilled water, was gavaged, and the other groups received only 2.00 mL of distilled water daily.³⁰ The MET gavage was performed only in the final 4 weeks of the 12-week period.

Exercise testing and training (HIIT and CT protocols). Exercise training was performed on a treadmill designed for rats. Before the exercise interventions started, the rats in exercise groups were familiarized with running on the treadmill (1 week, 5 days, and 10 min *per* day with a speed of 10.00 m min⁻¹).³² After the last familiarization session, Bedford *et al.* incremental exercise test³³ was used to evaluate maximum running speed, being standardized by Leandro *et al.* for Wistar rats.³⁴ Then, CT and HIIT were conducted for 12 weeks and five sessions *per* week (Tables 1 and 2). The entire exercise program for each exercise group was divided into three phases, including familiarization (before the start of main exercise routine), overload (weeks one to nine), and maintenance of work intensity (weeks 10 to 12). In the CT group, overload was implemented by increasing time and speed, while in the interval training group, overload was achieved by increasing the number of repetitions and speed. Exercise programs for both HIIT and CT groups were standardized to have similar intensities and distances each week. All exercise sessions were conducted in the morning, starting with a 5-min warm-up (speed of 10.00 m min⁻¹) and concluding with a 3-min cool down (speed of 10.00 m min⁻¹). In the 1st week, CT consisted of 20 min of

running at 40.00% of maximum oxygen consumption (speed of 18.00 m min⁻¹), being gradually increasing in intensity and distance until the 9th week when the exercise was performed for 33 min at 70.00% of maximum oxygen consumption (speed of 28.00 m min⁻¹). In the final 3 weeks, time and speed remained constant (Table 1). The HIIT in the 1st week included three repetitions of 4 min running at 60.00% of maximum oxygen consumption (speed of 24.00 m min⁻¹) and 2 min of active rest at 15.00% of maximum oxygen consumption (speed of 6.00 m min⁻¹). Intensity and repetitions were gradually increased, with overload continuing until the 9th week. The 9th week included five repetitions of 4 min running at 90.00% of maximum oxygen consumption (speed of 36.00 m min⁻¹) and 2 min of active rest at 30.00% of maximum oxygen consumption (speed of 12.00 m min⁻¹). Speed and repetitions remained constant in the final 3 weeks. To standardize the intensity and distance of CT and HIIT, a single bout of running with different durations was added to the end of repetitions (Table 2).

Sample collection, and lipid profile and MDA and hormone levels assays. To examine lipid profiles, once after 4 weeks of training, rats were anesthetized with an intraperitoneal injection of ketamine (100 mg kg⁻¹; Produlab Pharma BV, Raamsdonksveer, Netherlands) and xylazine (10.00 mg kg⁻¹; Produlab Pharma BV),³⁵ and blood samples were collected from the hearts of rats. Rats under-

went blood sampling were excluded from the study. After 8 weeks of training, the rats were anesthetized to examine their lipid profiles; blood was collected from their hearts, and these rats were also excluded from the study. Finally, after 12 weeks of training, at the end of the study, 48 hr after the last exercise session, and following a 12-hr fast, the rats were anesthetized, and blood samples were collected from their hearts. The blood samples were centrifuged at 3,000 rpm for 15 min; the serum was collected and stored at - 80.00 °C to examine lipid profile, MDA, luteinizing hormone (LH), and testosterone. Serum lipid profile, including total TG, LDL, HDL, and VLDL, was measured using the photometric method (Pars Azmoon, Tehran, Iran) by Selectra II autoanalyzer (Vital Scientific, Spankeren, Netherlands). The serum levels of hormones and MDA were measured using rat enzyme-linked immunosorbent assay kits (Biorbyt, Cambridge, UK) according to the manufacturers' instructions.

Statistical analysis. The SPSS Software (version 24.0; IBM Corp., Armonk, USA) was used for statistical analyses. The Kolmogorov-Smirnov test was employed to assess the normality of data. The significance level in this study was $p < 0.05$. To examine between groups and within groups differences, the one-way analysis of variance test was used. Tukey's *post hoc* test was utilized for pairwise comparisons. Graphs were drawn using GraphPad Prism (version 8.4; GraphPad Software Inc., San Diego, USA).

Table 1. Continuous training protocol.

Weeks	Time (min)	Speed (m min ⁻¹)	Distance (m)
1	20	18.00 (equal to 45.00% of VO ₂ max)	360
2	21	20.00 (equal to 50.00% of VO ₂ max)	420
3	24	20.00 (equal to 50.00% of VO ₂ max)	480
4	26	22.00 (equal to 55.00% of VO ₂ max)	572
5	28	22.00 (equal to 55.00% of VO ₂ max)	616
6	28	24.00 (equal to 60.00% of VO ₂ max)	672
7	30	24.00 (equal to 60.00% of VO ₂ max)	720
8	32	26.00 (equal to 65.00% of VO ₂ max)	832
9	33	28.00 (equal to 70.00% of VO ₂ max)	924
10	33	28.00 (equal to 70.00% of VO ₂ max)	924
11	33	28.00 (equal to 70.00% of VO ₂ max)	924
12	33	28.00 (equal to 70.00% of VO ₂ max)	924

VO₂: Maximal oxygen consumption.

Table 2. High intensity interval training protocol.

Weeks	Running		Active rest		Running added to the end of sets		Sets	Distance (m)
	Time (min)	Speed (m min ⁻¹)	Time (min)	Speed (m min ⁻¹)	Time (sec)	Speed (m min ⁻¹)		
1	4	24.00	2	6.00	85	24.00	3	360
2	4	26.00	2	8.00	135	26.00	3	420
3	4	26.00	2	8.00	0	0.00	4	480
4	4	28.00	2	10.00	85	28.00	4	570
5	4	28.00	2	10.00	190	28.00	4	618
6	4	30.00	2	12.00	190	30.00	4	672
7	4	30.00	2	12.00	0	0.00	5	720
8	4	34.00	2	10.00	85	34.00	5	831
9	4	36.00	2	12.00	140	36.00	5	926
10	4	36.00	2	12.00	140	36.00	5	926
11	4	36.00	2	12.00	140	36.00	5	926
12	4	36.00	2	12.00	140	36.00	5	926

Results

Lipid profile. Significant differences were observed in cholesterol, LDL, TG, HDL, and VLDL levels between groups ($p = 0.001$; Table 3). In the 1st measurement of cholesterol, LDL, TG, HDL, and VLDL levels, significant differences were observed between groups. Cholesterol, LDL, TG, and VLDL levels in PCOS group and all treated groups were significantly higher than control group, but HDL level in PCOS group and all treated groups was significantly lower than control group. However, there was no significant difference regarding cholesterol, LDL, TG, HDL, and VLDL levels between PCOS group and treated groups (Table 3). In the 2nd measurement of cholesterol, LDL, TG, HDL, and VLDL levels, significant differences were observed between groups. Cholesterol, LDL, TG, and VLDL

levels in PCOS group and all treated groups were significantly higher than control group, but HDL level in PCOS, PCOS + CT, and PCOS + MET groups was significantly lower than control group. Cholesterol and LDL levels in PCOS + CT, PCOS + HIIT, PCOS + MET + CT, and PCOS + MET + HIIT groups were significantly lower than PCOS group. There were no significant differences regarding VLDL and TG levels between PCOS group and treated groups. The HDL level in PCOS + HIIT, PCOS + MET+CT, and PCOS + MET + HIIT groups was significantly higher than PCOS group (Table 3). In the 3rd measurement of cholesterol, LDL, TG, HDL, and VLDL levels, significant differences were observed between groups. Cholesterol, LDL, TG, and VLDL levels in PCOS group and all treated groups were significantly higher than control group; however, HDL level in PCOS and PCOS + MET groups was

Table 3. Within-group and between-group differences in cholesterol, low-density lipoprotein (LDL), triglyceride (TG), high-density lipoprotein (HDL), and very-low-density lipoprotein (VLDL).

Parameters	Groups	1 st measurement	2 nd measurement	3 rd measurement	p within groups	p between groups
Cholesterol (mg dL ⁻¹)	Control	84.20 ± 9.36 [#]	87.40 ± 10.21 ^{#†}	86.80 ± 12.61 ^{#†}	0.885	0.001
	PCOS	138.00 ± 3.87 ^{*c}	149.60 ± 8.26 [*]	152.40 ± 9.58 ^{*a}	0.026	
	PCOS+CT	136.40 ± 5.31 ^{*c}	131.80 ± 5.93 ^{*#}	123.00 ± 9.13 ^{*#†a}	0.031	
	PCOS+HIIT	136.20 ± 7.08 [*]	130.80 ± 8.28 ^{*#†}	122.40 ± 12.23 ^{*#†}	0.108	
	PCOS+MET	139.40 ± 5.59 [*]	148.80 ± 9.78 [*]	148.00 ± 7.24 [*]	0.146	
	PCOS+MET+CT	136.80 ± 6.09 ^{*c}	130.60 ± 8.90 ^{*#†}	120.80 ± 5.35 ^{*#†a}	0.011	
	PCOS+MET+HIIT	136.60 ± 7.16 ^{*c}	131.00 ± 7.77 ^{*#†}	121.00 ± 9.77 ^{*#†a}	0.034	
LDL (mg dL ⁻¹)	Control	23.60 ± 5.77 [#]	25.40 ± 6.02 ^{#†}	25.80 ± 5.58 ^{#†}	0.818	0.001
	PCOS	79.00 ± 6.92 ^{*c}	87.80 ± 5.01 [*]	94.40 ± 12.07 ^{*a}	0.044	
	PCOS+CT	78.80 ± 5.80 ^{*c}	67.00 ± 9.92 ^{*#†c}	52.20 ± 7.69 ^{*#†ab}	0.001	
	PCOS+HIIT	73.00 ± 7.96 ^{*c}	59.60 ± 13.37 ^{*#†}	50.20 ± 7.82 ^{*#†a}	0.012	
	PCOS+MET	75.20 ± 6.45 [*]	86.20 ± 10.32 [*]	81.60 ± 11.41 [*]	0.234	
	PCOS+MET+CT	74.40 ± 5.12 ^{*c}	60.40 ± 7.63 ^{*#†}	52.00 ± 6.04 ^{*#†a}	0.001	
	PCOS+MET+HIIT	73.80 ± 10.84 ^{*c}	61.40 ± 6.54 ^{*#†}	50.40 ± 7.50 ^{*#†a}	0.003	
TG (mg dL ⁻¹)	Control	46.40 ± 5.41 [#]	50.20 ± 8.28 [#]	49.80 ± 10.29 ^{#†}	0.731	0.001
	PCOS	144.00 ± 11.18 ^{*c}	161.80 ± 10.42 [*]	169.60 ± 13.66 ^{*a}	0.015	
	PCOS+CT	148.40 ± 6.34 [*]	149.60 ± 10.83 [*]	149.60 ± 10.83 [*]	0.974	
	PCOS+HIIT	143.40 ± 6.94 [*]	144.80 ± 3.70 ^{*†}	144.20 ± 8.34 ^{*#}	0.946	
	PCOS+MET	151.00 ± 6.78 [*]	164.60 ± 14.32 [*]	152.00 ± 11.02 [*]	0.142	
	PCOS+MET+CT	142.20 ± 8.13 [*]	147.40 ± 3.50 [*]	144.20 ± 7.98 ^{*#}	0.504	
	PCOS+MET+HIIT	151.40 ± 8.32 [*]	145.60 ± 4.27 ^{*†}	144.20 ± 8.55 ^{*#}	0.294	
HDL (mg dL ⁻¹)	Control	51.80 ± 4.60 [#]	51.20 ± 7.08 [#]	50.60 ± 8.08 ^{#†}	0.961	0.001
	PCOS	28.40 ± 2.88 [*]	26.20 ± 4.08 [*]	23.20 ± 3.34 [*]	0.099	
	PCOS+CT	29.20 ± 3.03 ^{*c}	37.20 ± 5.76 [*]	41.80 ± 5.63 ^{*a}	0.006	
	PCOS+HIIT	32.00 ± 5.00 [*]	41.00 ± 5.52 ^{*#†}	41.40 ± 9.01 [#]	0.082	
	PCOS+MET	31.40 ± 3.50 [*]	28.20 ± 5.89 [*]	34.60 ± 6.02 [*]	0.200	
	PCOS+MET+CT	32.00 ± 2.91 ^{*bc}	43.00 ± 6.51 ^{*#†a}	40.60 ± 6.80 ^{*#a}	0.041	
	PCOS+MET+HIIT	31.00 ± 4.89 ^{*bc}	41.20 ± 4.91 ^{*#†a}	43.40 ± 6.26 ^{*#a}	0.008	
VLDL (mg dL ⁻¹)	Control	9.28 ± 1.08 [#]	10.04 ± 1.65 [#]	9.96 ± 2.05 [#]	0.731	0.001
	PCOS	28.80 ± 2.23 ^{*c}	32.36 ± 2.08 [*]	33.92 ± 2.73 ^{*a}	0.015	
	PCOS+CT	29.68 ± 1.26 [*]	29.92 ± 2.16 [*]	30.06 ± 2.13 [*]	0.950	
	PCOS+HIIT	28.68 ± 1.38 [*]	28.96 ± 0.74 ^{*†}	28.84 ± 1.66 [*]	0.946	
	PCOS+MET	30.20 ± 1.35 [*]	32.92 ± 2.86 [*]	30.40 ± 2.20 [*]	0.142	
	PCOS+MET+CT	28.44 ± 1.62 [*]	29.48 ± 0.70 [*]	28.84 ± 1.59 [*]	0.504	
	PCOS+MET+HIIT	30.28 ± 1.66 [*]	29.12 ± 0.85 ^{*†}	28.84 ± 1.71 [*]	0.294	

PCOS: Polycystic ovary syndrome; CT: Continuous training; HIIT: High-intensity interval training; MET: Metformin.

Between-group differences are marked with symbols. *: Difference with control group; #: Difference with PCOS group; †: Difference with PCOS + MET group. Within-group differences are marked with letters. ^{abc} Different letters indicate significant differences with the 1st, 2nd, and 3rd measurements, respectively.

significantly lower than control group. Cholesterol and LDL levels in PCOS + CT, PCOS + HIIT, PCOS + MET + CT, and PCOS + MET + HIIT groups were significantly lower than PCOS group. The TG level in PCOS + HIIT, PCOS + MET+CT, and PCOS + MET + HIIT groups was significantly lower than PCOS group. However, there was no significant difference regarding VLDL level between PCOS group and treated groups. The HDL level in PCOS + CT, PCOS + HIIT, PCOS + MET + CT, and PCOS + MET + HIIT groups was significantly higher than PCOS group (Table 3).

Body weight. Body weight was significantly higher in PCOS group compared to the control group. The body weight of all groups received intervention was lower than PCOS group, but this difference was statistically significant only in PCOS + MET + CT and PCOS + MET + HIIT groups (Fig. 1).

Testosterone, LH, and MDA levels. Serum levels of testosterone, LH, and MDA were significantly higher in PCOS group compared to the control group ($p = 0.001$; Fig. 1). The results of Tukey's *post hoc* test showed that serum levels of LH, testosterone, and MDA in PCOS + CT, PCOS + HIIT, PCOS + MET + CT, and PCOS + MET + HIIT groups had a significant decrease compared to the PCOS group, but there was no significant difference between PCOS and PCOS + MET groups (Fig. 1).

Discussion

One of the most common types of interval training is HIIT, consisting of intermittent near-maximal effort interspersed with periods of recovery and rest.¹⁵ These types of

physical activities vary in intensity, duration, rest period, number of repetitions, and other potential variables.³⁶ Evidence suggests that HIIT elicits physiological responses similar to those induced by moderate-intensity continuous exercise³⁷ and may even be superior in some aspects,³⁸ a fact inspiring research on this subject. For example, it has been observed that moderate-intensity continuous exercise leads to significant improvements in lipid profile and body weight, while HIIT is more effective and faster in improving these indicators.³⁹ In the present study, the serum cholesterol and LDL levels significantly decreased after 8 and 12 weeks of exercise alone or accompanied by MET. The TG level significantly decreased after 12 weeks of exercise alone or accompanied by MET, and no significant difference was observed between any of treated groups. However, VLDL level did not show significant difference after 8 and 12 weeks of exercise training. Finally, serum HDL level significantly increased after 8 and 12 weeks in groups undergoing either exercise alone or accompanied by MET, but no significant difference was observed between these groups.

Exercise training generally improves lipid profile by reducing TG and LDL levels and increasing HDL level during aerobic exercises.⁴⁰ Norepinephrine, epinephrine, and glucagon release fatty acids from stored TGs in adipose tissue. Norepinephrine and glucagon bind to specific receptors on the adipocyte membrane, activating adenylyl cyclase to produce cyclic adenosine monophosphate, and cyclic adenosine monophosphate then activates cyclic adenosine monophosphate-dependent protein kinase, leading to phosphorylation of hormone-sensitive lipase

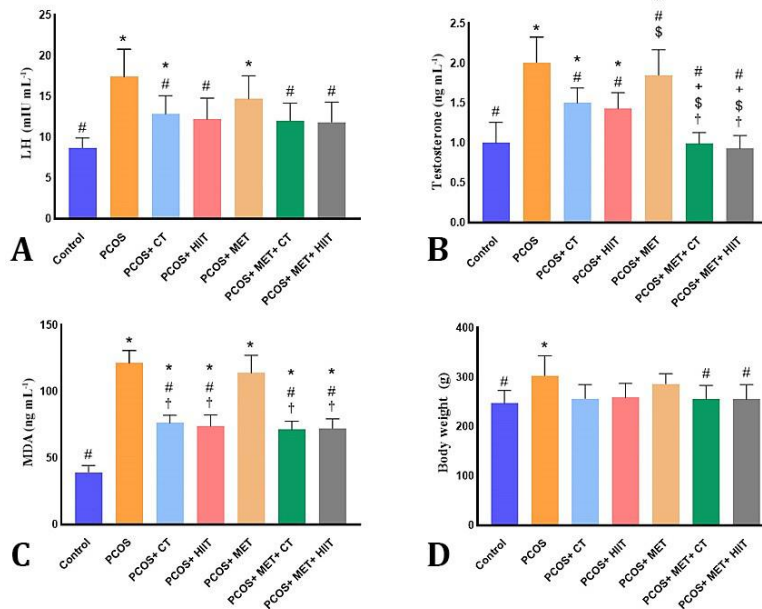


Fig. 1. Between-group differences in luteinizing hormone (LH), testosterone, and malondialdehyde (MDA) levels, and body weight. Between-group differences are marked with symbols. PCOS: Polycystic ovary syndrome; CT: Continuous training; HIIT: High-intensity interval training; MET: Metformin. *: Difference with control group; #: Difference with PCOS group; +: Difference with PCOS + CT group; †: Difference with PCOS + HIIT group; ‡: Difference with PCOS + MET group.

and perilipin. Phosphorylation of perilipin increases the activity of adipose TG lipase, providing more diacylglycerol substrates for hormone-sensitive lipase. The hormone-sensitive lipase then hydrolyzes diacylglycerol into free fatty acids and monoacylglycerols, and further hydrolysis is performed by monoacylglycerol lipase. Free fatty acids are transported to plasma membrane and bound to adipocyte fatty acid-binding protein, leaving adipocytes and binding to serum albumin. Exercise also induces lipoprotein lipase activity in skeletal muscle cells, increasing the hydrolysis of TGs from TG-rich lipoproteins, such as chylomicrons and VLDLs. Thus, free fatty acids, glycerol, free cholesterol, and phospholipids are released. Esterified cholesterol is also packaged into HDL particles and increases plasma HDL levels. Free fatty acids derived from lipoproteins and fat lipolysis are separated from albumin in blood, enter myocytes through specific fatty acid transporters, are transported to mitochondria, undergo beta-oxidation, and ultimately produce ATP, supplying the required energy.⁴¹

In study conducted by Cao *et al.* on PCOS in rats, a 6-week regimen of various exercises at different intensities revealed a significant increase in HDL levels and a decrease in total cholesterol levels. This effect was almost statistically comparable to the control group; however, LDL and TG levels decreased only in moderate-intensity CT and HIIT exercise groups compared to the PCOS group, and LDL reduction was more pronounced in HIIT group.⁴² In the present study, a significant improvement in lipid profile was observed after 8 or 12 weeks of CT or HIIT, but there was no significant difference between the two exercise methods. The obtained results may be due to the fact that in the present study, the researchers equated both types of protocols, CT and HIIT, based on intensity and distance. In a study by Ribeiro *et al.*, TG and LDL levels decreased after 16 weeks of continuous aerobic exercise, while these indicators remained unchanged in interval aerobic exercise. Additionally, total cholesterol and HDL levels did not show significant changes in both continuous and interval aerobic exercises. Different exercise protocols and different assessment methods may be the reason for inconsistent results.¹⁷

In the present study, serum TG level was improved only after 12 weeks of exercise, and beneficial effects of exercise on this indicator were shown by increasing duration of training, and as observed in the present study, increasing duration of exercise training led to better results in lipid profile. Regarding the impact of extended exercise duration on lipid profile of PCOS patients, Orio *et al.* divided overweight women with PCOS into two groups, including one engaged in 12 weeks of exercise followed by 12 weeks of inactivity, and other participated in 12 weeks of exercise followed by additional 12 weeks of exercise. The results demonstrated significant improvements in HDL and LDL levels after the initial 12 weeks of exercise in

both groups, with no significant differences between them. However, the continuous group with 12 additional weeks of exercise showed significant improvements in HDL and LDL levels compared to the group with only 12 weeks of exercise and baseline.⁴³ It has been suggested that high-intensity and long-term exercise protocols can increase changes in LDL particles (size and composition).⁴⁴

The MET is used in PCOS to improve metabolic abnormalities, lipid profile, androgen levels, and menstrual irregularities.²² In a recent study, it was found that use of MET in non-obese women with PCOS led to improvement of lipid profile.²³ Furthermore, 6-month treatment with MET significantly increased HDL levels in obese and non-obese women with PCOS, while LDL and TG levels remained unchanged in both groups. Total cholesterol levels increased in non-obese women but remained unchanged in obese women.²⁴ There is evidence that combining MET with exercise can offer additional advantages for exercise in PCOS patients.²⁵ The results of present study showed that 4-week treatment with MET alone had no significant effect on lipid profile, but groups receiving MET for 4 weeks along with exercise training did not show significant differences in lipid profile compared to the groups engaging in exercise training alone. The duration of MET usage might be one of the reasons for justifying the lack of significant differences in improving lipid profiles, and it is possible that if duration of MET usage is extended, differences between the groups may become apparent. According to this point, conducting more studies in this field and increasing the duration of MET intake along with exercise training can help to clarify this issue.

Increased LH and testosterone levels are the main symptoms of PCOS. Insulin resistance and increased LH levels in PCOS patients can lead to hyperandrogenism.⁴⁵ Also, the associations of ovarian hyperandrogenism with the oxidant-anti-oxidant status have been reported. Oxidative stress is demonstrated to be directly correlated with testosterone which may consequently contribute to hyperandrogenism in PCOS women. Oxidative stress is involved in altered steroidogenesis in the ovaries, thus contributing to increased androgen production, disturbed follicular development, and ultimately infertility.⁴⁶ Also, when comparing PCOS patients, overweight PCOS patients had higher levels of free testosterone and free androgen index.⁴⁷ Wu *et al.* observed that 12 weeks of aerobic exercise significantly reduced oxidative markers, including MDA, in patients with PCOS.²¹ In the present study, it was observed that MDA and LH levels and body weight decreased in the groups received exercise intervention alone or combined with MET compared to the PCOS group. Therefore, the reduction of these factors may have led to the reduction of testosterone levels and improvements in these treatment groups.

According to the results obtained in the present study, it was observed that exercise training was effective in improving the indicators measured in the present study, while MET alone did not have much effect. Also, when MET was used alongside exercise, no significant difference was observed compared to the exercise alone. Therefore, exercise training was an effective factor in improving most of the measured indicators, seemingly having a greater impact than taking MET alone.

Conclusively, our finding shows that HIIT and CT with longer durations with and without MET can be effective in improving serum lipid profile and MDA levels of patients with PCOS. In addition, no significant differences were observed between two exercise methods.

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Conflict of interest

The authors declare no conflict of interest.

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