

Brief Report

Effects of aerobic exercise on plasma lipoproteins in overweight and obese women with polycystic ovary syndrome

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Abstract

Background: Polycystic ovary syndrome (PCOS) is one of the most frequent endocrine disorders. The PCOS manifest by hyperandrogenism, hypertension and cholesterol and lipoprotein improper profiles. Changing the life style, e.g. increasing physical activities is the first approach in controlling PCOS.

Materials and Methods: Twenty-four women affected by polycystic ovary syndrome (PCOS) after medical screening were divided in to two groups: Experimental group ($n = 12$) and control group ($n = 12$), with the average age, weight, height, BMI and WHR of 26.87 ± 4.43 years, 75.71 ± 10.65 kg, 159.29 ± 6.44 cm, 29.86 ± 3.22 kg/m² and 91.75 ± 5.86 respectively. First the body composition such as BMI, WHR, percent body fat, weight and body fat mass were measured. In fasting blood samples the level of HDL, LDL, VLDL, triglyceride and cholesterol were measured. Then the experiment group underwent the effect of an aerobic exercise program. After 12 weeks, all the measured variables before intervention the test were re-measured. Correlated t-test was used for comparing the two groups before and after intervention the test and independent t-test was used for comparing the two groups ($P < 0.05$).

Results: The results showed that after 12 weeks of exercise, BMI, WHR, fat rate, weight and fat mass and triglyceride had significant reduction and HDL had significant increase. But no significant changes happened in LDL, VLDL, and cholesterol levels.

Conclusion: Reducing the weight by aerobic exercise in obese women and affected by PCOS can correct lipoprotein profile and improving health.

Key Words: Aerobic sport, body compositions, plasma lipoproteins, polycystic ovary syndrome

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder, affecting 8-12% of women^[1] and is one of the most prevalent causes of infertility in women.^[2] The disorder called Leventhal and Stein syndrome, the main clinical features of this syndrome are obesity, hyperandrogenism, menstrual cycle disorders and infertility occurs individually or

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simultaneously.^[3] Obesity and an ovulation is followed by cardiovascular disease, hypertension and altered cholesterol and lipoprotein profile.^[4] Reproductive outcome in women affected by PCOS intensely dependent on the body weight and metabolic condition.^[2] About 50% of women with polycystic ovary syndrome are overweight or obese defined by body mass index >25 or >30 kg/m.^[5] Abnormal lipoprotein pattern are prevalent disorders in PCOS^[6] and include increasing cholesterol, triglycerides and low-density lipoprotein (LDL) and the low level of high density lipoproteins (HDL).^[7] Central obesity is one of the prominent signs of this syndrome, found in different degrees (30-70%) and is directly related to insulin resistance.^[8-10] It is well known that obesity is associated with anovulation, pregnancy loss and late pregnancy complications (pre-eclampsia, gestational diabetes).^[11] The risk of developing PCOS rises with increasing obesity, as does the severity of insulin resistance, hyperinsulinemia, and ovulatory dysfunction, and the prevalence of metabolic syndrome, glucose intolerance, risk factor for cardiovascular disease, and sleep apnea.^[12] Direct correlation between PSA, hirsutism and hyperandrogenism state was shown in one study.^[13]

Recommended non-medical strategies for this group includes nutrition and physical activities.^[14,15] Reducing weight in obese patients is the first recommendation, since reducing weight causes reduction in insulin and androgen, as well as increase in SHBG.^[6] In addition to make better appearance of the patient, weight reduction could provide benefits in PCOS patients via improving the production of peripheral estrogen and the androgen condition in the ovary.^[12] By normalizing the factors such as BMI, waist and hip (WHR) size, systolic and diastolic blood pressure, blood sugar, triglyceride, cholesterol, lipoproteins (LDL and HDL), not only the emergence of cardiovascular diseases would be reduced, but it also causes survival with no cardiovascular disorders and improving life style.^[16] Reducing weight or doing exercise causes improvement in reproductive outcome in women with PCOS, but its mechanism is still unknown.^[17] Moderate activity incorporated into daily activities appears to be as effective in the reduction in diabetes risk and cardiovascular disease as that achieved with vigorous activity and may be more sustainable over the long-term.^[18] Physical exercise includes exercise for 3 days a week, each time for 20 min as for threshold stimulation for weight reduction and reducing body fat.^[19] As a primary strategy, the best solution is obtained by changing the life style that includes diets, exercises and behavioral interventions.^[17] In this regard, a research was done by^[20] 40 obese infertile women affected by PCOS, were randomized 20 women in diet group with high calorie diet (n = 20)

and moderate exercise programs group (n = 20) with the intensity of 60 to 70% VO₂ Max (Maximum oxygen volume) and physical exercises for 24 weeks. Hence in both groups, BMI, WHR and insulin with empty-stomach reduced, and a considerable improvement was observed in the menstrual cycle and fertility in both groups, but the ovulation rate and frequency of menstruation in the exercising group ($P < 0.05$) were higher than the control group.^[17] In a research, which the overweighted women with PCOS (n = 7) or without PCOS (n = 8) underwent an intense endurance exercise (one that involves the use of several large groups of muscles and is thus dependent on the delivery of oxygen to the muscles by the cardiovascular system) (1 h. 3 times/week) with no diets. Finally, the exercises reduced BMI, android mass and full fat mass; sensitivity to insulin was improved in all of the women. In the women with PCOS, AMH (Anti-müllerian hormone) was considerably less, in comparison with the control group, before and after the exercise, but no changes were observed in the women with PCOS for AMH. Since about 1.3% of the obese patients with PCOS are affected by glucose tolerance, and 7.5 to 10% are affected by diabetes type II, it seems that reducing the fat, especially viscera and intestinal fat has considerable effect in improving other biochemical risk factors.^[21] Women affected by PCOS have rates of total cholesterol, LDL and triglyceride and lower HDL.^[22] Also, hyperinsulinemia is more prevalent in overweighted women and its androgenic effects are more intensified.^[12] Physical activities or exercises reduce the fat mass, has deterministic role in increasing energy costs and effects the density of metabolic stress hormones (insulin, Cortisol, growth hormone, catecholamine, sex hormones, etc.) and metabolites (free fatty acids, lactic acid, triglyceride, etc.).^[23] Aerobic exercise is usually considered as a solution for facilitating weight reduction and improving lipidos compositions and serum lipoproteins.^[24] If factors such as BMI, waist and hip size, systolic and diastolic pressures, glucose, triglyceride, total cholesterol, lipoproteins with low density (LDL) and with high density (HDL) were normal, not only the development of coronary heart diseases reduce, but this could increase survival without the heart (cardiac) diseases and cause better life quality.^[25] Hence the preset research deals with the effect of aerobic exercises on plasma lipoproteins in women with polycystic ovary syndrome.

MATERIALS AND METHODS

The present study was of semi-empirical, pre-test, post-test type with a control group. The people selected for the research were among obese 20-30 year old women affected by PCOS, who had

gone to outpatient gynecologic clinics of Isfahan University and Shahinshahr. The diagnosis of PCOS was based on the based presence of biochemical or clinical hyperandrogenism and chronic an ovulation and sonography. Twenty-four women affected by PCOS were randomly selected in two groups of control and experimental, with BMI (29.86 ± 3.22) kg/cm², and average age of 26.87 ± 4.43 years [Table 1]. These people were not affected by cardiac, renal, liver diseases or diabetes and did not take medicines such as contraceptive pills, progesterone, metformin, clomiphene citrate, gonadotropins glitazone, as well as non-smoker or alcohol user.

They were not trained and had no regular exercises. The exercise included 12 weeks of aerobic exercise in moderate level (60 to 70% of maximum heart rate), for 3 sessions a week, each session for 60 min. The exercises included: Warning up: 10 min, main phase (aerobic exercise): 40 min and cooling down: 10 min. Polar Pulse measuring device from England was used to control the intensity of the exercises. Necessary notice was given to both groups regarding no change in their diet programs. Five milliliter of venous blood was taken from the left hand of the applicants by the lab. technician (all of the participants were recommended not to have oily food and carbohydrate-rich materials 12-14 h before doing the blood sampling and fasting. Two days after the blood sampling, the patients were analyzed on the analyzing device of body composition for determining their weight, BMI, WHR, body fat mass (the absolute body fat is the total weight of fat in the body) and percent body fat (body weight-lean body weight/body weight) $\times 100$ [Figure 1].

Terms of Use body composition analyzer equipment:

1. Lack of food and bathing before importantly test.
2. Temperature and humidity should be in the range.
3. Empty the digestive system is inactive.
4. No metal objects near the mobile device.
5. The people on the lowest clothes.
6. Giving accurate information to the device.
7. Full contact with the electrodes feet and

hands. 8. Presence of moisture in the palms of the hands and feet. 9. Standing on the balancing machine. 10. Putting forward head and hands beside the body with 15-20 degree angle. After entering the information into the devices after a few minutes of data were recorded on a raw form. Entire process measurements were performed by one person.

After 12 weeks of exercise, another test was taken from the patients (observing all the items in the pre-test). Two days after the blood sample collection of the 2nd phase, the patients underwent another analysis.

Study protocol was approved by the local ethics committee and all patients entered the study only after informed consent was obtained. Assays: Pars Azmoon kits and BT 3000 auto analyzers (Spain) were used for measuring blood lipoproteins (HDL, LDL, VLDL, TG, total cholesterol). Body composition analyzer equipment (3.0) (made in Korea) was used to determine body compositions. The height measuring device (SEGA, made in USA) was used for measuring the height of patient and polar S-Series toolkit (made in England) was used to determine the heart rate. Descriptive and inference methods were used for statistical analysis. For inner-group comparisons, correlation t-test and for inter-group analysis (Comparison of differences and post-test results), the independent t-test was applied. For analyzing the homogeneity of the variances of the comparing groups (control and experimental), Levene's test, and Kolmogorov-Smirnov test for controlling the normal distribution of the variables were used and for analyzing the data, SPSS software was utilized. The significance level for all the statistical analyses was considered at $P < 0.05$.

RESULTS

The distribution of clinical and biochemical diagnostic criteria of PCOS were compared to those of control subjects. Results of independent t-test for comparing the average subtraction of

Table 1: Physical specifications of the participants

Variable	Group	Number	Mean	Standard deviation	Confidence interval %95		Minimum	Maximum
					Too high	Too low		
Height (cm)	Test	12	159.08	5.57	155.55	162.62	152	168
	Control	12	159.50	7.32	154.85	164.15	145	169
Old (Years)	Total	24	159.29	6.36	156.61	161.98	145	169
	Test	12	26.33	4.50	23.47	29.19	18	35
	Control	12	27.42	4.36	24.45	30.19	22	35
	Total	24	26.88	4.37	25.03	28.72	18	35

BODY COMPOSITION ANALYSIS				InBody	
NAME	AGE	SEX	I. D.		
EXAM DATE :					
BODY COMPOSITION					
COMPARTMENT	MEASURED VALUE	TOTAL BODY WATER	SOFT LEAN MASS	LEAN BODY MASS	BODY WEIGHT
Intracellular Fluid (ℓ)					
Extracellular Fluid (ℓ)					
Protein Mass (kg)					
Mineral Mass (kg)		estimation			
Fat Mass (kg)					
MUSCLE - FAT DIAGNOSIS					
COMPOSITIONAL	UNDER	NORMAL	OVER		
Height (cm)	80% 85% 90% 95%	100% 105% 110%	115% 120% 125%		
Weight (kg)	60% 70% 80% 90%	100% 110% 120%	130% 140% 150%		
Soft Lean Mass (kg)	60% 70% 80% 90%	100% 110% 120%	130% 140% 150%		
Body Fat Mass (kg)	20% 40% 60% 80%	100% 160% 220%	280% 340% 400%		
Percent Body Fat (%)	Male 0% 5% 10% 15% 20% 25% 30% 35% 40% Female 8% 13% 18% 23% 28% 33% 38% 43% 48%				
Fat Distribution	Male 0.65 0.70 0.75 0.80 0.85 0.90 0.95 1.00 1.05 Female 0.60 0.65 0.70 0.75 0.80 0.85 0.90 0.95 1.00	WHR			
FLUID DIAGNOSIS					
SEGMENT	SEGMENTAL FLUID DISTRIBUTION (ℓ)			EDEMA EXAM	
	UNDER	NORMAL	OVER	Normal : 0.30 - 0.35	
Right Arm	40% 60% 80%	100% 120%	140% 160%		
Left Arm					
Trunk					
Right Leg					
Left Leg					
EVALUATION					
Muscle Type	Sarcopenic	Under	Weight Normal	Over	
	Proportionate	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
	Muscular	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Nutrition Status	Protein	Under	Normal	Over	
	Fat	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
	Mineral	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Upper Lower Balance	Arm	Developed	Normal	Undeveloped	
	Leg	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Right Left Balance	Arm	Balanced	Unbalanced		
	Leg	<input type="checkbox"/>	<input type="checkbox"/>		
WEIGHT CONTROL (kg)					
Target Weight					
Weight Control					
Fat Control					
Muscle Control					
FITNESS SCORE					
				Point	
CLASSIFICATION		NUTRITIONAL ASSESSMENT		BIOELECTRICAL IMPEDANCE	
<input type="checkbox"/> Cancer	<input type="checkbox"/> Surgical Patient	<input type="checkbox"/> Muscle Dystrophy			
<input type="checkbox"/> Strokes	<input type="checkbox"/> Rehabilitation	<input type="checkbox"/> Diabetes Mellitus			
<input type="checkbox"/> Pregnancy	<input type="checkbox"/> Nephropathy	<input type="checkbox"/> Osteoporosis			
<input type="checkbox"/> Obesity	<input type="checkbox"/> Hypertension	<input type="checkbox"/> Hyperlipidemia			
<input type="checkbox"/> Edema	<input type="checkbox"/> Arteriosclerosis	<input type="checkbox"/> Cardiovascular Disease			

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Figure 1: A sample form of wind instrument composition analysis of raw data

pre-test and post-test values for the variables of body compositions and plasma lipoproteins in the experimental and control groups, before and after 12 weeks of aerobic exercises are shown [Tables 2 and 3]. Accordingly, and following 12 weeks of aerobic exercise the difference of BMI rate ($P = 0.00$), WHR ($P = 0.01$), percent body fat ($P = 0.00$), body fat mass ($P = 0.00$), HDL ($P = 0.00$) and Triglyceride ($P = 0.02$) were significant between the two groups. But, the difference of LDL ($P = 0.01$), VLDL ($P = 0.2$) and cholesterol ($P = 0.97$) after 12 weeks of aerobic exercise were insignificant.

DISCUSSION

Our study result showed that body compositions: BMI, WHR, percent body fat, body fat mass reduced significantly after 12 weeks of aerobic exercise [Table 2]. The result was consistent with the findings of researches conducted by Samantha K *et al.*,^[26] Stener Victorin *et al.*,^[27] Vigorito *et al.*,^[28] Palomba *et al.*,^[21] Giallauria *et al.*,^[29] Thomson *et al.*,^[30] Bruner *et al.*,^[31] Randeva *et al.*,^[32] Brown *et al.*^[32] Exercise and physical activities reduce the body fat that stores estrogens and provides production of steroid hormones.^[33] The obtained results from the present research confirms

Table 2: Comparison between the groups for body compositions in the participants of both groups 12 weeks of aerobic exercise

Variable	Group	Mean	Standard deviation	t	Degrees of freedom	P	Result
BMI (kg/m ²)	Test	0.79	0.37	8.52	22	0.000	Significant
	Control	-1.18	0.45				
WHR	Test	1.33	1.61	2.99	22	0.01	Significant
	Control	-0.08	0.29				
Percent body fat	Test	3.04	1.98	5.58	22	0.000	Significant
	Control	-0.22	0.43				
Weight (kg)	Test	1.38	1.62	3.45	22	0.000	Significant
	Control	-0.1	0.30				
Body fat (kg)	Test	2.78	1.44	6.80	22	0.000	Significant
	Control	-0.21	-0.05				

Table 3: Comparison between the groups for plasma lipoproteins in the participants of both groups after 12 weeks of aerobic exercise

Variable	Group	Mean	Standard deviation	t	Degrees of freedom	P	Result
BMI (kg/m ²)	Test	0.79	0.37	8.52	22	0.000	Significant
	Control	-1.18	0.45				
WHR	Test	1.33	1.61	2.99	22	0.01	Significant
	Control	-0.08	0.29				
Percent body fat	Test	3.04	1.98	5.58	22	0.000	Significant
	Control	-0.22	0.43				
Weight (kg)	Test	1.38	1.62	3.45	22	0.000	Significant
	Control	-0.1	0.30				
Body fat (kg)	Test	2.78	1.44	6.80	22	0.000	Significant
	Control	-0.21	-0.05				

the effect of aerobic exercise in reducing the body compositions. The reductions in body compositions could be due to the lipolysis activity of the body adipose tissues.^[34] Long-term exercise increases lipolysis in the adipose tissue. This is approved by the microdialysis of the outer cell space of the subcutaneous adipose tissue. The main activator of lipolysis during exercise is sympathoadrenal system. Arner *et al.* by this method showed that inhibiting mechanism of alpha adrenergic regulates the resting lipolysis, while the stimulating effect of beta-adrenergic is important during the exercise. The effect of beta-adrenergic is based on sympathetic nerve system or epinephrine simulation. Epinephrine hormone is considered as the main activator of the sensitive lipase to hormones. But, there are also other hormones that simulate the lipolysis.^[35]

In a study with long-term exploitations, it was shown that in women with anovulation and having polycystic ovaries, the risk of D.M is 5 times more than control group with the same age.

Moreover, the insulin level in obese women with polycystic ovaries is higher than the lean women with polycystic ovaries and its androgenic effects are more severe. Also SHBG, LH level decrease significantly with aerobic exercise (with moderate activities). Reduction

of ovarian insulin level reduces the production of ovarian androgen. Normalizing the growth of follicle and its development and there is an assumption that it causes more ovulation.^[36] Since, WHR is the index for abdominal obesity and there are relations between WHR changes, body fat percentage and sensitivity to insulin, it can be said that by reducing WHR, and the rate of body fat after aerobic exercise, the average sensitivity to insulin increases and production of SHBG increases, too. By increasing SHBG, production of androgen decreases and finally, ovulation occurs.^[31]

Also the results of the present study showed the increase in HDL level and reduction of triglyceride after 12 weeks of aerobic exercise. But there were no significant changes in LDL, VLDL and total cholesterol levels [Table 3]. The result was consistent with the findings of researches conducted by Randeva *et al.*,^[31] Braner *et al.*,^[34] Vigorito *et al.*,^[27] Gialauria *et al.*,^[28] Staner Victorian^[26] and Samanta K *et al.*^[25]

In the study by Thompson *et al.*,^[29] a considerable improvement in cholesterol, HDL and LDL was observed following 20 weeks of exercise. Increasing HDL was in conformity with this research. It seems that intensity of the exercise and similar age in the participants is the reason for the conformity. Specific diet or difference in type, intensity and

duration of exercises in the research could be the reason for inconformity in other results. Various studies have shown that regular endurance exercise reduce cholesterol, triacylglycerol and LDL in blood, while increase the level of HDL. The decrease in triglyceride, cholesterol, VLDL and the increase in HDL after the exercise could justified through the following mechanism: The hydrolysis rate of triacylglycerol of VLDL increases after the exercise that is regarded due to increasing the activity of LPL enzyme of the muscles and increasing the section area of capillary endothelial after the exercise. In comparison to the inactive and stagnant people, the active people with endurance exercises have higher potentials for refining plasma triacylglycerol. This refinement could be dependent on increasing the activity of LPL enzyme of muscles and increasing the area of capillary capacity resulted by the exercise. By increasing of the two mentioned aspects, it could be expected that the rate of refining of VLDL and blood triacylglycerol to be increased.

Decomposition of triacylglycerol has close relations to synthesis of HDL, and hence any increase in the metabolic capacity, for metabolizing triacylglycerol could justify the higher value of HDL cholesterol in active people doing exercises. It seems that the reason for increasing HDL is due to increasing the production of HDL by liver and change in the activities of different enzymes, such as increasing LDL and LCAT (Lecithin-cholesterol acyltransferase) activities and reducing the hepatic lipase activity, followed by continuous sporting activities. Long term and intense exercise could create changes in LDL-C particles and their sizes and their compositions. Although the level of LDL-C changes continually, but exercise could reduce the LDL-C viscosity and increase larger, more floating LDL-C, and there is a relation that seemingly depends on the rate and intensity of exercise.^[34] The possible reason of increasing HDL is due to increasing the production of HDL in liver and changing the activity of different enzymes, such as increasing the activity of LDL and decreasing the activity of hepatic lipase, followed by the regular exercise activities. Regarding the relation of obesity and overweight in women with PCOS, with lipid profile abnormalities, hyperinsulinemia, diabetes type II and cardiovascular diseases, the present study could have beneficial effect in management and reducing the rate of complications in women with PCOS.

In most of the researches done, aerobic exercises cause the reduction in total cholesterol and triglyceride, LDL and VLDL, and increase in HDL, but in our research no significant decrease in the level of LDL, VLDL and

total cholesterol. Since apart from some, the rest of the participants in our research had normal levels of LDL, VLDL and cholesterol (which may be due to genetic aspects). The reason for did not reduce the variables during the 12-week of exercise could be due to the fact that the people usually have low levels of LDL, triglyceride and total cholesterol from the beginning. The relevant rates remain low during treatment by exercise, while in people with higher LDL, cholesterol and triglyceride, there would be a greater possibility for decreasing the relevant rates.^[19] The other possible reason is the inadequate difference in duration and intensity of the physical exercise for the variables mentioned before to be reduced.

Giallauria F *et al.*^[28] presented a research regarding the improvement of immune system independently by physical exercises, in women affected by PCOS. The participants in this research were 62 people in the experimental group (with average age of 22.8 and BMI of 29.2 kg/m²) and 62 people in the control group (with average age of 22.6 years and BMI of 29.5 kg/m²). The exercises of the exercise (sport) group included fixed cycling (3*30 min.week) with medium intensity (60 to 70% VO₂ max) that was done for 12 weeks and eventually the experimental group had a considerable reduction in BMI, and WHR, as compared to the control group. But there was no considerable change in the level of HDL and LDL. This was in conformity with the results obtained by the researcher. Giallauria used the fixed cycling for his exercise protocol, but we used aerobic exercises in our protocol despite similarity of duration and intensity of the exercise, but some discrepancies are observed in the results our research. Reducing of BMI and WHR and no alteration in LDL had conformity with the results we obtained, but no change in HDL was not in conformity with our results that indicate its increase.

In the research Tofighi *et al.*,^[33] 30 PCOS women were divided into experimental and control groups. The experimental group did selected aerobic exercises for 10 weeks, with the intensity of 50-75% of maximum heart rate. The results showed that the physical exercise reduced the rates of WHR, fat percentage, ratio of LH/FSH, SHGB and insulin serum of the experimental group, significantly, but no significant changes were observed for the values of BMI, testosterone serum. The only similar measured factor in the present research was the rate of BMI that it contradicts with the previous results. This could be due to the difference in the period of exercises.

In another study conducted by Thompson *et al.*,^[29] 94 patients were divided into 3 test groups that

included: First group with diet only (30 people); 2nd group will diet and aerobic exercises (31 people); and 3rd group with diet, aerobic exercises and endurance exercises. The duration and intensity of the exercises for the first weeks was 25-30 min and 60-65 maximum heart rate that reached to 45 min and 75-80 maximum heart rate. This research had the following results: A considerable reduction for BMI and insulin with empty stomach was observed for all the 3 groups and the fat mass was reduced in both of the exercise groups. Also a considerable improvement was reported in cholesterol and LDL. Ovulation and menstrual cycle were also improved in all the 3 groups. Reduction of BMI of this research was in conformity with the present research, but LDL levels contradict with our results. It is possibly due to different durations and intensities of the exercises in these researches.

By the end, increased risk of type 2 diabetes, dyslipidemia and cardiovascular disease had proven in women with PCOS.^[12] Lifestyle changes, had significant role in the prevention of complications. This study demonstrates that moderate intensity exercise (The exercise included 12 weeks of aerobic exercise, for 3 sessions a week, each session for 60 min), can improve several components of lipoprotein profiles. Our results support the beneficial effect of exercise and reducing the role of PCOS complications. Further, we recommend future interventional studies in this ground, involving exercise in prevention of metabolization in PCOS women.

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