ISSN: 2538-5887; Pathobiology Research. 2026; 29 (2): 15-21
DOI: 10.48311/mjms.2026.119853.82622

Improvement of myeloperoxidase and high-density lipoprotein following silymarin supplementation during aerobic training in adult women with metabolic syndrome

Review History:
Received: 2026-04-18
Revised: 2026-05-09
Accepted: 2026-06-01

Article Type:
Original Research

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ABSTRACT

Introduction: Recently, the role of antioxidant supplements during aerobic training has been highlighted to improve cardiovascular function in chronic metabolic diseases. In the present study, the effect of aerobic training with and without silymarin supplementation on myeloperoxidase (MPO) and High Density Lipoprotein (HDL) was evaluated in women with metabolic syndrome.

Methods: For this purpose, 48 adult females aged 30-40 years of old (30≤BMI≤36) with metabolic syndrome were randomly divided into control (no training), exercise (aerobic training, 8 weeks, 3 sessions/weekly), supplement (silymarin supplementation, 8 weeks, 280mg/daily) and combine (aerobic training+ silymarin). Fasting blood samples were collected before and 48 hours after lasting exercise session with regard to measuring serum MPO and HDL. Data were compared between groups using ANCOVA and Bonferroni Post-Hoc test ($p \leq 0.05$).

Results: Compared to control group, fasting HDL significantly increased in 3 intervention groups ($p<0.05$). Aerobic training with and without silymarin supplementation resulted in a significant decrease in MPO compared to the control group ($p=0.017$, $p<0.05$ respectively). Silymarin consumption alone did not affect MPO compared to the control group ($p=0.999$). Compared to the exercise and supplement groups, serum MPO was significantly deceased and HDL significantly increased in combined group ($p<0.05$).

Conclusion: With the conclusion from this article, aerobic training with and without silymarin supplementation leads to increased HDL in women with metabolic syndrome. This improvement may be attributed to a decrease in MPO in response to these interventions. Understanding the underlying mechanisms responsible for these changes requires further studies in this area.

Keywords: Exercise, High-density lipoprotein, Silymarin, cardiovascular function, Metabolic syndrome

Introduction

Metabolic syndrome is a set of metabolic disorders that often include symptoms of cardiovascular diseases, type 2 diabetes, and obesity (1,2,3). As abdominal obesity, insulin resistance, hyperglycemia, increased blood pressure and cardiovascular risk factors, and decreased high-density lipoprotein have been reported as its symptoms (4). In other words, metabolic syndrome is associated with some abnormalities that are mainly known as cardiovascular risk factors. As abdominal obesity (waist circumference more than 88 and 102 in women and men, respectively), increased blood glucose levels (fasting glucose higher than 100 mg/dL), high blood pressure (systolic higher than 120 and diastolic higher than 90 mmHg), and lipid disorders are among the symptoms of metabolic syndrome. These abnormalities are also often associated with hyperinsulinemia, glucose intolerance, atherogenic dyslipidemia (decreased high-density lipoprotein (HDL) (below 40 and 50 mg/dL in men and women, respectively) and increased triglycerides (above 150 mg/dL), free fatty acids, and very low-density lipoprotein (VLDL) (3).

On the other hand, obesity and metabolic syndrome lead to endothelial dysfunction by damaging the vasodilating properties of the endothelium, which in turn is the first step in the prevalence or progression of cardiovascular diseases (5). Metabolic syndrome also through

factors such as blood vessel constriction, increased insulin resistance and sympathetic activity, hyperactivity of the renin-angiotensin system can be leads to vascular dysfunction and hypertension (6). Although the mechanisms of the relationship between metabolic syndrome and endothelial dysfunction are not yet well defined, studies on obese populations have pointed to the effective role of vascular proinflammatory factors and oxidative stress on endothelial dysfunction under these conditions. Studies on laboratory mice fed a high-fat diet have revealed that endothelial dysfunction occurs in response to a decrease in the capacity of the antioxidant system and an increase in the activity of NF-kB-dependent inflammatory pathways (7). Therefore, coronary artery occlusion and atherosclerosis are observed within the vascular surface.

Meanwhile, the lack of oxidants with antioxidants or oxidative stress and the increase in reactive oxygen species or free radicals in the presence of obesity and metabolic syndrome are also associated with endothelial function damage (3, 8). For example, myeloperoxidase (MPO), a cationic homodimeric glycoprotein with a molecular weight of 15 kDa, as a member of the heme peroxidase enzyme family, plays a role in the innate immune system and in combating microbial pathogens under physiological conditions, but its increase in pathological conditions or oxidative stress, such as obesity-related metabolic disorders, plays a very important role in inflammatory processes and oxidative stress (9).

Possible mechanisms of action of MPO in the occurrence or increase of cardiovascular diseases are possible through factors such as stimulation of Low-density lipoprotein (LDL) conversion to atherogenic oxidized form, alteration of apolipoprotein A-1, inefficient HDL production (reduction of reverse cholesterol transport ability by HDL), dysfunction of endothelial cells, formation of vulnerable plaques and abnormal ventricular remodeling after myocardial infarction (10). Based on the aforementioned evidence, MPO is introduced as an independent risk factor for predicting cardiovascular function through its effect on HDL and LDL levels (11). Therefore, it seems that the improvement of its systemic levels in response to therapeutic stimuli is associated with an increase in vascular endothelial function. Recently, exercise alone or in combination with antioxidant supplements has been proposed to improve oxidant or antioxidant factors effective in cardiovascular function. However, despite the fact that a decrease in MPO following aerobic exercise in women with metabolic syndrome was reported by Valeh *et al* (2023) (12), Shemsheki *et al* (2013) reported no change in MPO following 8 weeks of resistance training in inactive women (13). On the other hand, Naseri-Rad *et al* (2020) reported an increase in HDL along with a decrease in leptin and Triglyceride (TG) following 10 weeks of aerobic exercise in men with metabolic syndrome (3). The antioxidant or anti-inflammatory effects of exercise training in obese individuals or those with obesity-related diseases have also been reported by some other researchers (14,15).

Independent of exercise training, some studies have supported the effectiveness of silymarin as an antioxidant supplement on oxidative and antioxidant stress factors (16). In this context, it has been noted that the consumption of silymarin by inhibiting MPO synthesis in diabetic rats leads to improvements in cardiovascular risk factors such as increased HDL and decreased TG, TC, and LDL (17). Silymarin refers to a set of flavonolignan compounds (seven types of flavonolignans: silylin type A, silylin type B, isosilin type A, isosilin type B, silychristin, isosilicristin and silydianin and one taxifolin) found in the seeds of the milk thistle plant with the scientific name Silybun marianum with antioxidant properties, and scientific studies have supported its role in reducing free radicals and inhibiting lipid peroxidation and increasing Superoxide dismutase (SOD) (18). Regarding the cardiovascular effects of silymarin, it can be noted that reducing cholesterol absorption and its consequence, reducing cholesterol and LDL, while significantly increasing HDL, are also beneficial effects of silymarin (19). Other confirmed effects of silymarin include a reduction in malondialdehyde as a marker of oxidative stress (18) and an increase in antioxidant capacity (20). However, its effect on MPO as a marker of vascular function has not been studied. Based on this limitation and the lack of studies reporting the simultaneous effect of exercise training and silymarin on MPO and lipid profile indices in women with metabolic syndrome, the present study aimed to investigate the effect of silymarin consumption during aerobic training on serum levels of MPO as well as HDL in women with metabolic syndrome.

Materials and Methods

Subjects

The statistical population of this study consists of adult women with metabolic syndrome. Given the nature of the study which was quasi - experimental and field-laboratory and also based on the sample size in some previous studies (12, 21,22,23), the case sample is 48 adult women with metabolic syndrome aged 30-40 years of old who were selected from the statistical population and randomly divided into 4 groups: control (no intervention), exercise (8 weeks of aerobic training, 3 days/weekly), supplement (2x140 mg silymarin tablets daily, 8 weeks), combination (aerobic training + silymarin, 8 weeks). The number of samples in each group is 12 people (May 2025, Islamshahr, Iran).

Inclusion and exclusion criteria

Obesity ($30 \leq BMI \leq 36$) and a history of metabolic syndrome for at least the past 3 years are the main inclusion criteria for the study. The study subjects were non-smokers and had no history of controlled diet or regular exercise for at least the past 6 months. People with cancer, gastrointestinal diseases, and orthopedic abnormalities were excluded from participating in the study. The study subjects also did not use any nutritional supplements or medications during the past 6 months except for temporary illnesses. Failure to participate in continuous exercise training, as well as failure to use silymarin or other nutritional supplements effective in cardiovascular function or antioxidant agents and oxidative stress during the study were exclusion criteria. Observation of any persistent metabolic and digestive discomfort caused by supplement use during the study were also exclusion criteria. Any injury caused by exercise training was also an exclusion criterion.

Anthropometric measurements and blood sampling

The height of the subjects was measured using a wall-mounted height gauge with an accuracy of 0.1 cm. Before and after intervention, weight and body fat percentage were measured using a body composition measuring device (OMRON, Finland). Body mass index was calculated by dividing weight (kg) by height (m2). Then, after 10-12 hours of overnight fasting, the subjects came to the laboratory between 8-9 am for blood sampling, and 5 cc of blood was taken from the brachial vein (pre-test). The subjects were asked to refrain from any heavy physical activity for 48 hours before blood sampling. 48 hours after the last exercise session, blood sampling was repeated in the same manner as the pre-test (post-test). Serum MPO was measured by the calorimetric method using a specialized kit (Navand Salamat Company, Iran), and HDL was measured by the calorimetric method using laboratory kits from Pars Azmoun Company (Iran).

Aerobic intervention and silymarin supplementation

Subjects in the supplement group consumed 2x140 mg silymarin tablets daily under the pharmaceutical name Livergol (morning and evening) produced by Gol Daru Company for 8 weeks. Subjects in the exercise group participated in an 8-week aerobic training (3 days/weekly). In the combination group, subjects performed 8-week aerobic training and consumed 2x140 mg silymarin tablets daily during the period.

Aerobic training was performed at an intensity of 50 to 70% of maximum heart rate. Each exercise session lasted 30 to 50 minutes and consisted of a warm-up, main exercise, and a 10-minute cool-down. The main exercise began with 20 minutes of 50 to 55% of maximum heart rate in the first and second weeks, and with a 5% increase in exercise intensity every two weeks and 2 minutes in exercise time each week, it reached an intensity of 65 to 70% of maximum heart rate and a duration of 40 minutes at the end of the eighth week (5).

Table 1: Distribution of exercise intensity while running during the training program

weeks	Exercise intensity (%HRmax)	Time of running
First and second	$\%50 \leq$ intensity $\leq \%55$	3 × 7.5 minute
Third and fourth	$\%55 \leq$ intensity $\leq \%60$	4 × 8 minute
Fifth and Sixth	$\%60 \leq$ intensity $\leq \%65$	4 × 9 minute
Seventh and eighth	$\%65 \leq$ intensity $\leq \%70$	4 × 10 minute

Table 2: Pre and post intervention of anthropometrical indexes for all groups (M ± SD)

Variable	Time	Control	Exercise	Supplement	Combine
Weight (kg)	pre	85 ± 3.31	85.21 ± 1.92	84.96 ± 1.89	85.67 ± 2.52
	post	85.1 ± 3.27	82.04 ± 1.57	84 ± 1.86	81 ± 2.11
	p-value	0.504	0.001	0.001	0.001
AC (cm)	pre	98 ± 2.90	97.42 ± 1.98	98.85 ± 1.55	98.33 ± 2.27
	post	98.25 ± 3.02	91.83 ± 1.75	97.42 ± 1.56	92.83 ± 2.08
	p-value	0.082	0.001	0.001	0.001
BMI (k/m2)	pre	32.21 ± 0.90	31.84 ± 0.60	31.73 ± 1.10	31.88 ± 0.38
	post	32.24 ± 0.93	30.66 ± 0.66	31.37 ± 1.09	30.15 ± 0.67
	p-value	0.481	0.001	0.001	0.001
BF (%)	pre	39.86 ± 1.05	40.03 ± 1.10	39.81 ± 1.35	40.38 ± 0.86
	post	39.93 ± 1.13	35.35 ± 1.80	38.76 ± 1.29	35.19 ± 1.10
	p-value	0.202	0.001	0.001	0.001

AC; abdominal circumference, BMI; body mass index , BF; body fat percentage
Data compared by paired t-test

Table 3: Pre and post intervention of hematological markers for all groups (M ± SD)

Variable	Groups	Pre-test	Post test	*p-value (paired t test)*
MPO	Control	29.08 ± 2.93	29.15 ± 3.55	0.947
	Exercise	28.38 ± 2.21	25.68 ± 2.31	0.001
	Supplement	28.53 ± 1.90	27.57 ± 2.52	0.035
	Combine	28.46 ± 2.94	22.73 ± 3.23	0.001
HDL	Control	39.58 ± 1.96	38.94 ± 1.96	0.361
	Exercise	39.61 ± 2.04	42.38 ± 2.03	0.001
	Supplement	39.27 ± 1.64	41.99 ± 1.48	0.001
	Combine	39.37 ± 2.03	46.13 ± 1.77	0.001

MPO*; Myeloperoxidase,* HDL*; High-density lipoprotein*

Statistical Methods

Statistical comparisons were made in SPSS version 22. One-way ANOVA was used to compare serum levels of dependent variables between the study groups. To compare the data between the groups, ANCOVA test was used along with Bonferroni's post hoc test. P value of less than 0.05 was regarded as indicative of a significant difference. Paired t-test was used to determine within-group changes of each variable in groups.

Results

The within-group changes in each of the anthropometric indices are summarized by paired t-test in table 2.

Based on the one-way ANOVA test, non-significant difference was observed in baseline (pre-test) serum MPO (p = 0.452) and HDL (p = 0.563) between the groups. The within-group changes in serum MPO and HDL are summarized by paired t-test in table 3.

Based on the findings of the ANCOVA test, a significant difference was observed in MPO change between groups (p=0.009). The results of the Bonferroni post hoc test for serum MPO are summarized in Table 4. Based on the data in this table, compared to the control group, serum MPO in the exercise and combined groups decreased significantly. Also, no significant difference was observed between the exercise and supplement groups. On the other hand, a significant difference was observed in serum MPO between the combination group and the exercise and supplement groups. In other words, serum MPO in the combined group decreased significantly compared to the exercise and supplement groups.

The findings of the ANCOVA test also indicate a significant difference in HDL changes between the study groups ($p<0.05$). The results of the Bonferroni post hoc test are summarized in table 4. Based on the data in this table, compared to the control group, HDL increased significantly in the exercise, supplement, and combined groups. Also, no significant difference was observed between the exercise and supplement groups. On the other hand, a significant difference in HDL was observed between the combine group and the exercise and supplement groups. In other words, serum HDL in the combine group increased significantly compared to the exercise and supplement groups.

Table 4: Results of the Bonferroni post hoc test for MPO and HDL between study groups

Group	Group	MPO		HDL *(p-value)*	
		Mean difference *(p-value)*		Mean difference *(p-value)*	
Control	Exercise	2.877	0.017	- 3.422	0.001
Control	Supplement	1.128	0.999	- 3.199	0.001
Control	Combine	5.908	0.001	- 7.292	0.001
Exercise	Supplement	- 1.758	0.354	0.224	0.999
Exercise	Combine	3.021	0.011	- 3.870	0.001
Supplement	Combine	4.779	0.001	- 4.093	0.001

Discussion

The main finding of the present study is a significant decrease in MPO following aerobic training with and without silymarin supplementation in women with metabolic syndrome. In other words, compared to the control group on which no intervention was performed, 8 weeks of aerobic training alone and combined with silymarin consumption led to a significant decrease in MPO as one of the effective oxidant factors in cardiovascular function in women with metabolic syndrome. In such a way that the aforementioned interventions also led to a significant increase in HDL in the study groups compared to the control group. Regarding the effect of exercise training on MPO levels, although limited studies have been conducted. However, Shemsheki *et al* (2011) have pointed out that it did not change following resistance training in inactive women (13). Gharah Dashkhany *et al* (2023) have also reported no change in MPO following TRX training in obese women even in the presence of a significant increase in total antioxidant capacity (23). The lack of change in MPO in the aforementioned studies is reported while Valeh *et al* (2023) reported a significant decrease in MPO following aerobic exercise in women with metabolic syndrome (12). Ojaghi *et al* (2021) also reported a decrease in serum MPO accompanied by an increase in antioxidant function and a decrease in MPO in the heart tissue of rats with cardiac ischemia following progressive endurance exercise (24). Although the differences in the findings in the aforementioned studies may be attributed to the type of exercise or the type of population studied, the findings of the present study indicate an improvement in both MPO and HDL following aerobic exercise, which in a way indicates the effectiveness of this exercise method on cardiovascular function with an emphasis on improving MPO levels in women with metabolic syndrome.

This is while the increase in MPO by stimulating the conversion of LDL to its atherogenic oxidized form and its inhibitory effect on reverse cholesterol transport by HDL or oxidative changes of HDL leads to impaired vascular endothelial function and the formation of vulnerable plaques after myocardial infarction (10). MPO also, by participating in the respiratory burst reaction and with the help of H2O2, causes the production of multiple oxidants and the conversion of H2O2 to HOCL, which leads to oxidative damage to cells and tissues (25). Its harmful effects on the heart and blood vessels have been confirmed by reducing the induction of nitric oxide (NO) production and, as a result, impaired vascular endothelium function. Also, the production of various reactive oxidants through myeloperoxidase has a significant impact on the prevalence of inflammatory processes caused by the immune system and tissue damage caused by inflammation, such as atherosclerosis (25). Increased MPO also acts as a stimulus in the production of some reactive oxidant species such as chloramine, hypochlorite, and tyrosine radicals, which oxidize proteins, lipids, and HDL (26).

Despite the inhibitory effect of aerobic training on MPO in the present study, its levels did not change in the supplemented group compared to the control group. In other words, 8 weeks of silymarin consumption did not lead to a change in serum MPO levels compared to the control group. However, its consumption during aerobic exercise in the combined group led to a significant decrease in MPO compared to the group that had only aerobic exercise. In other words, silymarin supplementation in women with metabolic syndrome who had participated in aerobic exercise not only reduced MPO levels compared to the control group but also reduced its levels compared to the group that had only experienced aerobic exercise. It is noteworthy that the changes in HDL in the aforementioned

groups were also in line with MPO. This evidence may somehow point to a reduction in the inhibitory effect of MPO on HDL in response to aerobic training in the presence of silymarin consumption. In confirming the mobilizing effect of silymarin on the effectiveness of aerobic exercise on cardiovascular function, we can refer to the findings of Roghani *et al* (2012), who reported an increase in HDL and a decrease in TG, TC, and LDL, and an improvement in the oxidative stress profile in diabetic mice following silymarin consumption (17).

The antioxidant, cardiovascular, and antidiabetic effects of silymarin alone or in combination with exercise training have also been reported in some other studies. As Mahmoodi *et al* (2018) reported a decrease in glucose, glycosylated hemoglobin and insulin resistance in type 2 diabetics following 8 weeks of aerobic training combined with silymarin consumption (21). Hassani *et al* (2013) also reported a decrease in MDA in response to 6 weeks of endurance training combined with silymarin consumption in inactive men (22). The aforementioned evidence supports the antioxidant, endothelial, and cardiovascular effects of silymarin during aerobic training in women with metabolic syndrome. Although the improvement of both HDL and MPO following silymarin consumption during aerobic training is considered one of the strengths of the study, the vascular effectiveness of these interventions cannot be indicated by measuring these variables alone, and the lack of measurement of other components of vascular function such as NO, VEGF and MDA or antioxidant indices is a limitation of the study, which is recommended for evaluation in future studies.

Conclusion

Given the inhibitory effect of MPO on HDL, the improvement in HDL in response to aerobic training may be attributed to the reduction in MPO following this intervention. On the other hand, although silymarin consumption by these patients does not directly affect HDL, its consumption during aerobic training leads to a greater effectiveness on MPO and HDL than exercise alone, and this evidence emphasizes the use of silymarin during aerobic training in women with metabolic syndrome with the aim of improving cardiovascular function.

Acknowledgments

The authors wish to thank the Islamic Azad University of Islamshahr Branch For their support and assistance.

Funding

This research was funded by Islamic Azad University, Islamshahr Branch (IR.IAU.PIAU.REC.1403.003).

Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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