The effect of aerobic training with silymarin consumption on lipid profile in men with type 2 diabetes

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Abstract
Background and objective: Type 2 diabetes (T2D) is a metabolic disease that leads to increased levels of lipids, therefore the present study aimed to investigate the effect of eight weeks of aerobic training (AT) with silymarin (S) consumption on lipid profile in men with type 2 diabetes.

Methods: 60 middle-aged male volunteers were randomly divided into 4 groups, including: (1) control (C) + placebo, (2) AT + placebo, (3) S consumption and (4) AT+S consumption. The AT groups performed for eight weeks, three sessions per week and 20-45 minutes per session at an intensity of 60 to 85% of the reserve heart rate, and the S groups of consumed 140 mg / kg of S daily (in two meals). Serum levels of Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), Cholesterol (CHO), and Triglyceride (TG) were measured before and after the test. Data were analyzed using one-way ANOVA and Bonferroni’s test, and P value less than 0.05 was considered significant.

Results: AT reduced serum levels of LDL, CHO, TG and increased HDL in men with T2D(P ≥ 0.05). Consumption of S reduced LDL, CHO, TG and increased HDL in men with T2D(P ≥ 0.05). AT and S consumption reduced LDL, CHO, TG and increased HDL; also, decrease in LDL, CHO, TG and increase in HDL in the AT and S consumption group was more favorable than the effect of S alone (P ≥ 0.05).

Conclusion: It seems that AT and S consumption simultaneously have interactive effects on reducing LDL, CHO, TG and increasing HDL in men with T2D.

Keywords: Aerobic Training, Silymarin, Lipid Profile, Type 2 Diabetes

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Introduction

Diabetes mellitus is a metabolic disease characterized by hyperglycemia due to lack of access to insulin or reduced sensitivity to it, or a combination of both (1). Numerous risk factors such as increased lipid profiles, blood pressure, and glycosylated hemoglobin increase the risk of cardiovascular disease in diabetic patients (2). T2D is caused by increased insulin resistance due to obesity and sedentary lifestyle and so reduced insulin secretion from beta cells. This disease is associated with abnormal levels of fats and lipoproteins, including LDL, very low-density lipoprotein (VLDL), CHO, and TG (3). Various factors such as genetic background, diet, and reduced physical activity are among the most important risk factors for T2D(4). Many researchers around the world are trying to use various methods to prevent or treat diabetes or reduce the effects of diabetes.

Today, the use of herbs and extracts to treat diseases has increased (5). S is the most important active ingredient in the scientifically called plant Silybum marianum, which is made up of a group of chemicals called flavonolygenates (6). S has cholesterol-lowering and blood sugar-lowering effects and antioxidant and anti-inflammatory effects (7). Studies have shown that S significantly reduces CHO absorption and consequently reduces LDL and increases blood HDL (8). The anti-diabetic properties of S have also been confirmed in patients with T2D and in the experimental model of diabetes mellitus in recent years (9). One of the suggested methods to reduce the complications of diabetes, including cardiovascular complications, is physical activity with diet and medication (10).

Over the past few years, researchers have suggested that weight loss and lifestyle changes are the first steps in treating and preventing this type of disease (11). AT is a common type of exercise in the treatment of people with T2D, which improves glycemic control, lipid status, reduces body fat and lowers fasting blood glucose (12).

Research has shown that eight weeks of AT improves fat profiles in women with T2D (13). The American Diabetes Association recommends that patients with T2D should perform at least 150 minutes of moderate-intensity AT or 90 minutes of high intensity aerobic activity in a week (14). The results of a review study showed that moderate to high intensity AT for 12 weeks reduced LDL, CHO and TG (15). Smart et al. in a meta-analysis study in 2018 found that exercise improved fat profile, reduced fat mass, and insulin resistance (16).

So far, few studies have looked at the effects of S supplementation on the profile of T2D men, and due to the lack of adequate research on the interactive effects of AT and S consumption in diabetic men, the aim of this study is to investigate the effect of eight weeks of AT with S consumption on the lipid profile of T2D men.

Materials and Methods

The present study was performed on type 2 diabetic men in Gachsaran city with pre-test - post-test design and control group after obtaining the ethics license in the research of Islamic Azad University of Gachsaran branch and registration in Iran Clinical Trial Center with registration number IRCT202002150446506N3. Inclusion criteria in the study included being male sex, aged 35 to 50 years and fasting blood sugar between 130 and 250 mg / dL, and exclusion criteria...
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comprised having other chronic diseases, smoking in the past six months, having regular exercise in the last 6 months, diabetic complications such as diabetic foot ulcers, etc., which cause the inability to perform physical activity. All subjects competed the consent form. Subjects were randomly divided into 4 groups, including: (1) C + placebo (2) AT + placebo (coated tablet containing wheat flour) (3) S supplementation and (4) AT + S consumption. The AT groups performed training for eight weeks, three sessions per week and each session for 20-45 minutes at an intensity of 60 to 85% of the heart rate reserve (17).

S receiving groups consumed two coated tablets containing 140 mg / kg S daily (one after breakfast and one after dinner) by the Livergol brand manufactured by GolDarou Company (economic code IRC12280250372) (18).24 hours before the start and end of the study, with 12 hours of fasting, 5ml of blood was taken from the brachial vein and the levels of LDL- C, HDL- C and total CHO were measured by enzymatic method through ParsAzmoon Company kit of Iran and photometric method; also, total CHO was measured by enzymatic method.

Statistical analysis

Initially, the findings of the research in the pre-test and post-test were presented in the descriptive statistics in terms of mean and standard deviation. The Kolmogrov-Smirnov test was used to check the normal distribution of the findings. For inferential analysis of research findings, dependent sample t-test was used to investigate changes in the pre-test and post-test. To examine between-group differences, differences in the pre-test and post-test were calculated. Also, the differences in changes were analyzed using one-way ANOVA and Bonferroni’s post hoc test. Findings of this study were analyzed by SPSS software version 22 at a significance level of 0.05.

Results

The demographic characteristics of the subjects are presented in Table 1.

The results of dependent sample t-test showed that there was no significant difference in the pre-test and post-test levels of LDL (P =0.089), HDL (P =0.077) and CHO (P =0.44) and TG (P =0.101) in the C group; however, posttest levels of LDL (P =0.001), CHO (P =0.001), TG (P =0.001) in the S consumption, AT and AT + S groups were significantly lower than pre-test, and HDL levels in all three groups of intervention (P = 0.001) was significantly higher than the pretest (Figures 1 to 4).

Table 1. Demographic Characteristics of the Subjects Before and After Intervention (Mean±SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (Year)</th>
<th>Height (cm)</th>
<th>Weight (kg) Pretest</th>
<th>Weight (kg) Posttest</th>
<th>WHR Pretest</th>
<th>WHR Posttest</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>46.58 ± 1.51</td>
<td>171.43 ± 0.44</td>
<td>78.35 ± 4.03</td>
<td>78.32 ± 4.11</td>
<td>2.31 ± 92.68</td>
<td>± 2.54 93.38</td>
</tr>
<tr>
<td>S</td>
<td>46.08 ± 1.82</td>
<td>172.34 ± 0.40</td>
<td>80.09 ± 4.88</td>
<td>77.48 ± 4.88</td>
<td>93.37 ± 1.56</td>
<td>92.92 ± 1.85</td>
</tr>
</tbody>
</table>
| AT    | 46.59 ± 1.89 | 172.61 ± 0.47 | 79.62 ± 5.23 | 76.96 ± 5.13 | 93.32 ± 1.82 | 90.46 ± 1.64 *¥
| AT+S  | 47.00 ± 1.73 | 172.58 ± | 79.22 ± 2.74 | 75.89 ± 2.84 | 93.63 ± 1.57 | 90.68 ± 1.64 *¥

The results showed significant differences in LDL (P =0.001, F=15.98), HDL (P =0.001, F=7.89), CHO (P =0.001, F=10.81) TG (P =0.001, F=9.55) in the research groups.

The results of post hoc test showed that the blood levels of LDL in the S (P = 0.040), AT (P = 0.001) and AT + S (P = 0.001) groups were significantly lower than group C. Blood levels of LDL in the AT + S group (P = 0.040) were significantly lower than in the S group, but there was no significant difference between the LDL blood levels in the AT + S (P = 0.732) and AT groups and between the AT (P =0.308) and the S groups (Figure 1).

In addition, the results of post hoc test showed that the blood levels of HDL in the S (P = 0.043), AT (P = 0.008) and AT + S (P = 0.001) groups increased significantly compared to group C. Also, there was no significant difference in the blood levels of HDL in the AT + S (P =0.223) with group S and the AT + S (P = 0.566) with group AT as well as group AT (P =0.914) with group S (Figure 2).
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Figure 2. HDL levels in the four research groups. Data are presented as mean ± SEM. Statistical analyses were performed using two-way ANOVA with Bonferroni’s post hoc tests. +++ (P = 0.001) Significant increase compared to the pre-test
#Significant increase at P ≥ 0.05 and ### Significant decrease at P ≥ 0.05 compared to group C

The results of the post hoc test showed that the blood levels of CHO in the S (P =0.008), AT (P =0.001) and AT+ S (P =0.001) groups were significantly lower than group C. The levels of CHO blood in the AT + S group (P =0.332) were not significantly different from the S and AT + S (P =0.584) groups, and the CHO levels in the AT group (P =0.554) were not significantly different from S (Figure 3).

Figure 3. CHO levels in the four research groups. Data are presented as mean ±SEM. Statistical analyses were performed using two-way ANOVA with Bonferroni’s post hoc tests. +++ (P = 0.001) Significant decrease compared to the pre-test
##Significant increase at P ≥ 0.05 and ### Significant decrease at P ≥ 0.05 compared to group C

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The results of the post hoc test showed that the blood levels of TG in the S (P = 0.007), AT (P = 0.001) and AT + S (P = 0.001) group were significantly lower than group C. The levels of blood TG on the AT + S (P = 0.479) group did not differ significantly from group S and in the group AT + S (P=0.898) from group AT. Also, the TG levels in the group AT (P = 0.875) were not significantly different from group S (Figure 4).

![Figure 4](image_url)

**Figure 4.** TG levels in the four research groups. Data are presented as mean ± SEM. Statistical analyses were performed using two-way ANOVA with Bonferroni’s post hoc tests. +++(P = 0.001) Significant decrease compared to the pre-test
###Significant decrease at P = 0.001 compared to group C.

**Discussion**

The most common lipid disorder in patients with T2D is high blood serum triglyceride levels and low HDL, which is a risk factor for metabolic syndrome and is one of the most important causes of cardiovascular disease in these patients (19).

The results of the present study showed that aerobic training reduced LDL, CHO, TG and increased serum HDL in men with T2D. The results of the present study are consistent with the results of Saghebjoo, et al., Shakil-ur et al. (20,21) and inconsistent with the results of Adogu et al. And Lade et al. (22,23). Probably reason for lack of consistency can be the type of subjects, the type of sports activity, the intensity and volume of sports activity or nutrition. Many studies have shown that different types of exercise have an effect on blood lipids.

A study of diabetics found that 3 months of aerobic activity increased HDL blood levels and decreased CHO, TG and LDL in T2D patients compared to the diabetic control group (20). The reasons for increased HDL can be augmented LPL (Lipoprotein Lipase) enzyme activity as a result of exercise. In fact, LPL enzyme is effective in converting HDL to VLDL, and with increasing activity, HDL levels increase. On the other hand, LCAT (Lecithin - Cholesterol Acyltransferase), in addition to LDL, converts CHO to HDL particles.

Increasing this enzyme may be responsible for increasing HDL due to exercise. LCAT has been shown to be greatly increased in some exercises. Exercise also appears to
increase lipolysis and reduce fatty acids in the muscles themselves (24). Studies have shown that aerobic activity increases the fat catabolism and reduces TG in diabetic patients by increasing the volume of mitochondria and the activity of lipase protein enzymes during the activity and during recovery (25). Other findings show that taking S reduces LDL, CHO, TG and increases serum HDL in men with T2D which are consistent with the results of the research of Ebrahimpour et al. 2018 and Giuseppe et al. 2017 (26,27) and inconsistent with the results of the study of Khalili et al. 2017 and Famouri et al. 2017 (28,29). The reasons for the inconsistency of the results can be the dose of the drug, the duration of the drug and the type of test.

Ebrahimpour et al. concluded that daily consumption of 140 mg of S improves the glycemic index and lipid profile of T2D patients. Mohammadi et al. 2019 reported that S extract, like other plant extracts, contains flavonoid compounds that, in addition to strong antioxidant properties, stabilize cell membranes and increase cell glutathione, which may affect fat metabolism (30).

Daily administration of two 140 mg S capsules twice for 12 weeks reduced insulin resistance index and improved fat profile in the experimental group compared to the C group (31). Several mechanisms have been proposed regarding the beneficial effect of S on blood lipids. One of them is sibilin, which is one of the compounds in S, which can reduce CHO synthesis by inhibiting hydroxy 3 - methylglutaryl coenzyme A reductase. 3 - Hydroxy 3 - Coenzyme Methyl Glutaryl A is a speed-limiting enzyme reductase in CHO biosynthesis (32). Using the same mechanism, statin drugs are used to lower CHO (33).

The results of the present study showed that AT and consumption of S reduced LDL, CHO, TG and increased serum HDL in men with T2D. Also, lowering LDL, TG, and increasing HDL in blood serum in the AT and S consumption group were more desirable than S consumption alone.

There have been limited studies on the effect of concurrent consumption of S and training on serum levels of fat profiles. The results of a study on training rats showed that S use by decreasing the levels of TG and serum lactate, decreasing protein kinase A expression, phosphovanol pyruvate carboxykinase, Peroxisome proliferator-activated receptor gamma (PPARg) in the liver tissue and decreasing AMPK expression, increasing expression of dehydrogenase kinase 4 (PDK4) pyruvate in muscle tissue increased beta-oxidation of fatty acids from the mentioned pathways (34). In another study, researchers found that eight weeks of endurance training combined with S consumption reduced fat peroxidation (18). Given that in the present study, the effect of AT along with S consumption on some parameters was more than training and S alone, it can be concluded that these two interventions together can interactively improve the fat profile in diabetic patients. Regarding the limitations of the present study, the small size of the sample can be mentioned, which is considered a limitation. Undoubtedly, further research and a bigger sample size are essential to increase the reliability of our findings. Other limitations of the study were the lack of complete monitoring of the diet throughout the course; also, other non-athletic physical activities were not fully monitored. A more detailed study is needed in the future to better understand the mechanisms involved in the results observed in this study. Therefore, it is recommended that a similar study be
performed with careful control of the diet and physical activity of the subjects.

Conclusion

It seems that AT combined with S consumption can improve the fat profile in patients with type 2 diabetes; Therefore, it is recommended that patients with T2D should use both of these interventions simultaneously to improve metabolic indicators.

Authors' contributions

All authors contributed equally to this work.

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