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# Effect of 4 Weeks HIIT with Spirulina Supplementation Intake on Plasma Total Antioxidant Capacity (TAC) and Lipid Peroxidation (MDA) in Women with Type 2 Diabetes

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## Abstract

**Objective:** Oxidative stress plays a key role in the pathogenesis of type 2 diabetes mellitus (T2DM) and its complications. Exercise and anti-oxidant supplements are two potential approaches to delay the development of T2DM. The purpose of this study was to evaluate the interaction effects of spirulina supplementation and high intensity interval training (HIIT) on oxidative stress and total antioxidant capacity in inactive women with T2DM.

**Materials and Methods:** This research was a quasi-experimental study with pretest-posttest control group design. Our study subjects were 55 women with T2DM (age of 51.95 ± 5.57 years and BMI of 30.55 ± 4.63 kg/m$^2$) that were randomly divided into 4 groups: 1- exercise and spirulina (n= 15), 2- spirulina (n= 15), 3- placebo (n= 15), 4-control (n= 10) without exercise and supplementation. Participants received 2 grams spirulina supplement per day. Training program included three sessions pre-week walking and running on a treadmill for 4 weeks, each session consisted of 10 minutes of warming and 10 minutes of cooling with a 50-70% HRR intensity and 25 minutes of HIIT (The training interval of 4-minute sections with 85-95 % HRR intensity and 3-minute active rest sections, with 50-70 % HRR intensity). All evaluations were performed with SPSS statistical software using analysis of covariance to assess between-group differences and t-test to assess within-group differences.

**Results:** Our study results showed that the plasma level of MDA decreased significantly in the exercise + placebo group compared to the control group ($P$= 0.03). However, the level of TAC was not changed significantly in our experimental groups compared to the control group ($P$= 0.7).

**Conclusion:** Based on the findings of this study the spirulina supplementation and HIIT can be good stimuli for reducing oxidative stress in women with T2DM.

**Keywords**: Exercise, Spirulina supplement, Malondialdehyde, Total antioxidant capacity

QR Code

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## Introduction

Type 2 diabetes (T2DM) is one of the causes of premature death in certain medical conditions, such as cardiovascular disease (CVD), and neuropathy, retinopathy, and kidney disease (1). T2DM is a complex metabolic disorder characterized by hyperglycemia due to the body's inability to produce insulin or use its full potential (2). Today, the role of oxidative and free radical stress in the pathogenesis of T2DM has received more attention and study has shown that hyperglycemia increases oxidative stress (3). Besides, it has been shown that the body's antioxidant capacity for counteracting the production of free radicals is strongly associated with diabetes intensity and its side effects (4). Consequences of free radicals are include decreased glucose transport channels, decreased insulin secretion from pancreatic β cells, protein breakdown and oxidation, DNA damage, free fatty acid production, and increased vascular permeability that can cause and exacerbate diabetes (5). Lipid peroxidation is a well-known component for oxidative stress caused by ROS, and for its quantitative measurement, MDA measurement is a suitable method (6).

Regular exercise and physical activity may be effective in improving insulin resistance by improving insulin function and vascular function (by increasing biological nitric oxide) as well as increasing ROS-detoxification and reducing ROS production (7). High-intensity interval training (HIIT) is a type of exercise that involves repetitive periods of intense exercise that are separated by rest periods (8).

While exercise-induced oxidative stress is thought to be beneficial for optimal tissue function and adaptation to physiological stress, the effect of intensity of exercise on the physiological oxidative stress is not yet clear (9,10). Parker, McGuckin (11) showed that intense exercise (70%⁄ VO2max) significantly increased plasma TAC compared to low-intensity exercise. In the other study, Zwetsloot, Nieman (12) showed 80% increase in GPx activity and a 60% reduction in MDA levels during 4 weeks of intense physical activity.

In a variety of physiological and pathological conditions, such as strenuous exercise, altitude physical activity, and diseases such as diabetes, the internal antioxidant system cannot fully and quickly cope with these conditions. Therefore, the antioxidant role of dietary supplements is especially important in such situations. Spirulina is a microscopic cyanobacterium (green water algae) and a rich source of protein and vitamins, especially B12, minerals, carotenoids, and phycocyanin (13).

This supplement is an important source of a pigmented protein called phycocyanin C, which has anti-inflammatory and antioxidant properties. The beneficial effects of spirulina supplementation on antioxidant levels have been investigated and confirmed by Ismail, Hossain (13) in patients with chronic pulmonary obstruction (COPD) and diabetic mice by Gargouri, Magné (14).

Another study confirms the effects of this herbal supplement on anemia, increased production of antibodies, prevention of infection, modulation of blood sugar and lipids, and protection of the liver (15). In addition, spirulina can help control inflammation and oxidative stress caused by exercise, which may reduce exercise-induced fatigue (16). Hernández-Lepe, López-Díaz (17) used a cross-sectional cross-study study to control 80 healthy overweight and obese individuals and found that regular physical activity and a balanced diet reduced weight gain in obese people, as well as heart risks and lowers metabolism and possibly increases antioxidant capacity. Considering a few studies on the effect of spirulina supplementation and HIIT in patients with T2DM, this study aims to examine the effect of HIIT with spirulina supplementation on plasma MDA and TAC levels.



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## Materials and Methods

This research was a quasi-experimental study with pretest-posttest control group design.

### Participants

Our study participants were 55 individuals with T2DM who were selected according to medical documents and were approved by a specialist doctor. The sample size was calculated using G*Power version 3.1.9.7 for a significant difference between the two groups, with a power of 80% and an alpha value of 0.05 based on the study by McGuckin et al. Therefore, the required sample size n=50 subjects was obtained.

Participants were selected by referring to diabetes centers and other medical centers in Shahroud city. Our study participants have a mean age of 51.95 (± 5.57) years and a body mass index of 4.75(± 4.63) kg / $m^2$. Our study inclusion criteria were; a history of T2DM more than 6 month and less than 10 years, insulin dependence, ages from 40 to 65 years, and the use of common diabetes medications such as Metformin and Glibenclamide, lack of any cardiovascular disease, and lack of high blood pressure. Exclusion criteria were included; Lack of regular participation in training programs and lack of cooperation due to personal reasons or despises.

Prior to the start of the study, during a briefing session, the participants' consent form was signed and the patient's medical history was collected by the Public Health Questionnaire and their medical records. All volunteers participated in a training program on how to properly perform exercises and how to take supplements.

One of the goals of the briefing session (training course) was to reduce the participants drop by familiarizing with the training capacity of participants, because some of the participants may not be able to complete the training program due to physical limitations/pain. Participants were matched by BMI, blood sugar and age and then was randomized to 4 groups: 1- Intense interval exercise + Spirulina supplementation (15 people), 2- Intense interval exercise + taking placebo (15 people), 3- Spirulina supplementation (15 People) and, 4- Control

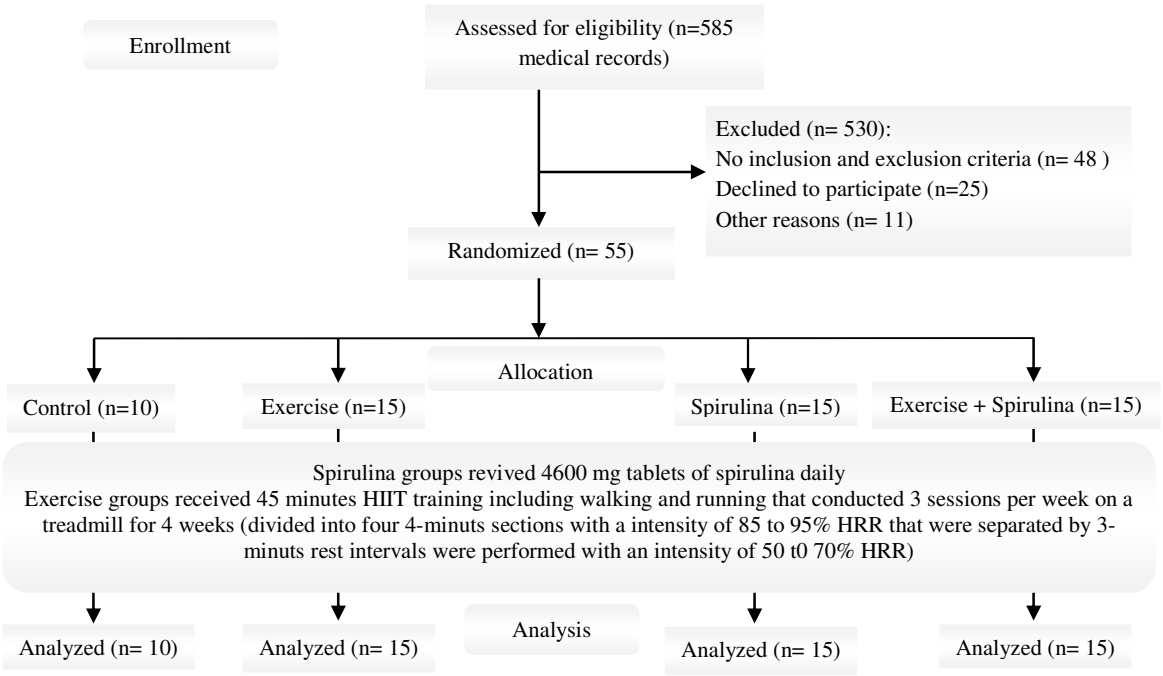



**Figure 1. The consort flow diagram**



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(10 people without exercise and Spirulina supplementation and only taking placebo).

Both groups (Group 1 and Group 2) were implemented in the HIIT program. Our study training protocol was conducted in 3 sessions per week, walking and running on a treadmill for 4 weeks. The training sessions lasted 45 minutes and each session began with 10 minutes of warm-up that performed with 50 to 70 %HRR intensity and ended with 10 minutes of cooling again performed with 50 to 70% of HRR intensity. The training sessions were divided into four 4-minute sections with an intensity of 85 to 95% HRR. Training sessions were separated by 3-minute rest intervals that were performed with an intensity of 50 to 70% HRR(18). To control the exercise intensity, the participant's heart rate was controlled during exercise. The following Karvonen Formula was used to determine the reserve heart rate:

Age - 220= maximum heart rate

Reserve heart rate = Rest heart rate - Maximum heart rate

Target heart rate = Rest heart rate + heart rate reserve × (exercise %intensity)

In addition to HIIT program, group 1 took 4500 mg tablets of spirulina daily. Group 3 took only 4500 mg tablets of Spirulina daily (19).

Participants' blood sample consisted of 10 ccs of blood from the arm vein that was sampled one day before the first session of the exercise (pre-test) and 48 hours after the last session of training in the fourth week (post-test). Participants' blood sample was gathered after 10 to 12 hours of fasting between 8 p.m. 10 a.m. Blood samples were centrifuged at 3000 rpm for 14 minutes, then serum was isolated and frozen, and stored at -70. C. The serums were transferred to the laboratory of Bu Ali Research Institute in Mashhad for measurement. All outcomes were measured before the training protocol (pre-test) and after 4 weeks of the post-test exercise program.

Body composition was measured using Inbody 230 machine (made in Korea) and finger blood sugar was measured by easy gluco fingerprint test (made in Korea).

Systolic and diastolic blood pressure was performed with a mercury blood pressure monitor (ALPK2 -300V; Tanaka Sangyo Co., Ltd., Tokyo, Japan) before and after the training protocol.

Plasma Malondiode aldehyde (MDA): MDA was measured using the ELISA method by a commercial chemical calorie testing laboratory kit based on the manufacturer's protocol (MDA test kit; ZellBio GmbH, Ulm, Germany). This method can determine MDA with a sensitivity of 0.1 micro mols. The intra-rater and inter-rater coefficient of this test was 5.8% and 7.6%, respectively.

Plasma Total Antioxidant Capacity (TAC): The total antioxidant capacity was measured using the ELISA method by a commercial kit (ZellBio GmbH, Ulm, Germany). TAC was measured based on a 490 nm colorimetric oxidation measurement method. The TAC level was examined as the amount of antioxidant in the sample, which was compared with the action of ascorbic acid as a standard. This method can determine the TAC with a sensitivity of 0.1 mm (100 µmol / L). It is claimed that the internal variation between the test is less than 3.4% and 4.2%.

Prior to the start of the study, during a briefing session, the participants' consent form was signed and the patient's medical history was collected by the Public Health Questionnaire and their medical records. All volunteers participated in a training program on how to properly perform exercises and how to take supplements.

One of the goals of the briefing session (training course) was to reduce the participants drop by familiarizing with the training capacity of participants, because some of the participants may not be able to complete the training program due to physical limitations/ pain. Participants were matched by BMI and then was randomized to 4 groups: 1- Intense interval exercise + Spirulina supplementation (15 people), 2- Intense interval exercise + taking placebo (15 people), 3- Spirulina supplementation (15 People) and, 4- Control

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(10 people without exercise and Spirulina supplementation and only taking placebo).

In addition to exercise, group 1 took 4500 mg tablets of spirulina daily. Group 3 took only 4500 mg tablets of Spirulina daily (19). Participants' blood sample consisted of 10 ccs of blood from the arm vein that was sampled one day before the first session of the exercise (pre-test) and 48 hours after the last session of training in the fourth week (post-test). Participants' blood sample was gathered after 10 to 12 hours of fasting between 8 p.m. 10 a.m. Blood samples were centrifuged at 3000 rpm for 14 minutes, then serum was isolated and frozen, and stored at -70. C. The serums were transferred to the laboratory of Bu Ali Research Institute in Mashhad for measurement. All outcomes were measured before the training protocol (pre-test) and after 4 weeks of the post-test exercise program.

Descriptive statistical and inferential statistical methods were used for data analyses. Shapiro Wilk's statistical test was used to determine data distribution, and the Leven test was used to evaluate the variance homogeneity. The T-test was used to assess within-group differences (before and after), the analysis of covariance (ANCOVA) was used to assess between-group differences, and the Bonferroni test was used to identify the differences between which groups. All evaluations were performed with SPSS statistical software (version 22.0 for Windows, SPSS Inc., Chicago, IL, USA) and the significance level was 0.05.

## Ethical considerations

All steps were performed according to the instructions of the ethics committee of Shahroud University of Medical Sciences and the IRCT20180711040425N1 trial code.

## Results

Forty-seven participants completed the research. Spirulina consumption was safe and no specific side effects have been reported during the study period. Clinical features and descriptive statistics of participants of each group are presented in Table 1.

The results of one-way ANOVA showed that there was no significant difference between groups in the pretest regarding height (*P*= 0.73), weight (*P*= 0.78) and body mass index (*P*= 0.24), indicating homogeneity of groups in terms of individual characteristics (Table 2).

Also, there were no significant difference between groups at the pretest regarding; MDA (*P*= 0.14), TAC (*P*= 0.58), systolic blood pressure (*P*= 0.9), and diastole blood pressure (*P*= 0.9) (Table 3).

The results of the covariance analysis to assess the differences in plasma levels of MDA and TAC between groups are shown in Tables 2 and 3, respectively. This test showed a significant difference between groups in the plasma level of MDA (*P*= 0.03) (Table 3).

In addition, the plasma level of TAC (*P*= 0.7) did not show a significant difference

**Table 1. Clinical and biochemical characteristics of research groups in baseline**

| Variables | Exercise + Spirulina Mean (±SD) | Exercise Mean (±SD) | Spirulina Mean (±SD) | Placebo Mean (±SD) | *P* |
|---|---|---|---|---|---|
| **Age (y)** | 50.08 (±4.46) | 51.08 (±6.01) | 53.36 (±6.50) | 54.43 (±4.35) | 0.3 |
| **Height (cm)** | 157.92 (±8.83) | 154.83 (±2.94) | 157.36 (±4.05) | 156.14 (±9.22) | 0.73 |
| **Weight (kg)** | 75.5 (±10.6) | 71.67 (±10.42) | 73.57 (±10.61) | 73.35 (±5.54) | 0.79 |
| **BMI (kg/m$^2$)** | 31.4 (±6.1) | 30.23 (±4.1) | 29.7 (±4.2) | 30.4 (±3.2) | 0.54 |
| **Systolic blood pressure (mm Hg)** | 12.8 (±1.49) | 11.71 (±0.69) | 11.90 (±1.51) | 11.79 (±1.11) | 0.899 |
| **Diastolic blood pressure (mm Hg)** | 8.20 (±0.69) | 8.17± (0.54) | 8.04 (±0.88) | 8.02 (±0.50) | 0.894 |

**Table 2. Plasma MDA and TAC for research groups in baseline**

| Variables | Exercise + Spirulina Mean (±SD) | Exercise Mean (±SD) | Spirulina Mean (±SD) | Placebo Mean (±SD) | *P* |
|---|---|---|---|---|---|
| **MDA (µm/L)** | 8.80(±36.4) | 9.76 (±41.9) | 72.14 (±38.05) | 57.24 (±20.28) | 0.138 |
| **TAC (µm/L)** | 0.58(±0.031) | 0.543 (±0.036) | 0.553 (±0.034) | 0.595 (±0.034) | 0.83 |

Abbreviations: MDA: Malondialdehyde, TAC: Total antioxidant capacity



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between groups (Table 4). The results of the Bonferroni test showed that the difference between the groups at the MDA level was due to the difference between the exercises + supplement group and the exercise + placebo group with the control group.

## Discussion

Some symptoms associated with T2DM, such as; Hyperglycemia, insulin resistance, and dyslipidemia can be caused by a variety of mechanisms, such as advanced glycation end products (AGEs), inflammation, increased polyol pathway flux (glucose-to-fructose conversion pathway), increased hexosamine pathway flux, and increased mitochondrial superoxide production that caused to oxidative stress (19). There is ample evidence that biological antioxidant defenses are degraded in diabetics' individuals (20). Oxidative damage in people with diabetes can be reduced by taking antioxidants (21).

These antioxidant and protective effects can be due to phycocyanins, β-carotene, and other vitamins and minerals found in spirulina (22), which are specifically involved in reducing lipid peroxidation (23). In this regard, Kim, Cheong (24) stated using spirulina may be useful for protecting cells from lipid peroxidation and DNA oxidative damage. A study by Lee, Park (25) on 37 patients with T2DM after using an 8 g of spirulina supplementation over 12 weeks achieved similar results. Contrary to our study, Shyam, Singh (26) reported that GSH, MDA, TAS, and SOD were unchanged in healthy individuals after taking 1 gram spirulina per day for 30 days. Possibly these contradictory results can be due to dose differences and the participants' characteristics (healthy people in the Shyam et al study). Consist of this study, Gordon, Morrison (27) also showed that malondialdehyde concentration, the product of lipid peroxidation, and indicator of oxidative stress were significantly reduced in patients with T2DM.

Given that exercise combined with spirulina supplements has significant effects on blood pressure, glycemia, and lipidemia, it can be effective in preventing and controlling diabetes side-effects (28). Increased oxygen consumption, as occurs during strenuous exercise, may increase the body's ROS production capacity, which can lead to increased oxidative stress and subsequent lipid peroxidation (29). Therefore, prescribing spirulina supplements can protect the body by reducing lipid peroxidation and reducing the production of free radical derivatives, which indicates a decrease in plasma MDA levels and normalization of GSH and SOD levels (30). In line with our results, Kalpana, Kusuma (29) study showed that spirulina supplementation resulted in a significant decrease in MDA levels inducted during exercise. Gauze-Gnagne, Lohoues (31) also examined the effect of spirulina antioxidant effect on marathon runners and observed that secondary oxidation levels were reduced to half by spirulina consumption. Kalafati, Jamurtas (32) also found that spirulina supplementation reduced MDA and increased TAC in participants. In this study, the dose of spirulina was 6 grams per day for 4 weeks.

**Table 3. Plasma MDA data for research groups (mean ±SD)**

| Groups | Pre-test | Post-test | *P* |
|---|---|---|---|
| **Exercise + Spirulina** | 83.3 (±36.4) | 44.8 (±15.5) | 0.03 |
| **Exercise** | 94.76 (±41.9) | 43.3 (±16.7) | |
| **Spirulina** | 68.5 (±37.6) | 41.1 (±16.7) | |
| **Placebo** | 57.24 (±20.3) | 77.04 (±26.9) | |

**Table 4. Plasma TAC data for research groups (mean ±standard deviation)**

| Groups | Pre-test | Post-test | *P* |
|---|---|---|---|
| **Exercise + Spirulina** | 0.58 (±0.11) | 0.60 (±0.10) | 0.74 |
| **Exercise** | 0.54 (±0.12) | 0.55 (±0.13) | |
| **Spirulina** | 0.53 (±0.13) | 0.59 (±0.14) | |
| **Placebo** | 0.59 (±0.20) | 0.58 (±0.20) | |



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On the other hand, exercise is a strong producer of ROS, and research has shown that increased exposure to ROS leads to adapt the antioxidant enzyme in the heart muscle and skeletal tissues in animal models (33). Some studies have shown that performing HIIT have similar metabolic effects to aerobic activity on glucose control and many clinical complications of diabetes (34). On the other hand, there is a direct relationship between exercise intensity and changes in total antioxidant capacity (TAC) (11).

Findings from several studies have also confirmed that strenuous exercise over time may lead to beneficial changes in homeostasis (increased TAC) and greater health benefits than low- and moderate-intensity exercise (35). In the same vein, Cipryan (36) and Fisher, Schwartz (35) also reported an increase in antioxidant indicators (CAT and GPX) after intense periodic exercise. Wuorinen, Page (37) observed the highest levels of TAC in the intense exercise group during the 10 weeks after exercise by examining the various acute and chronic effects of exercise intensity on TAC. Increased antioxidant levels due to the extra oxygen consumed during regular exercise training lead to oxidative stress (38). Our study results show that none of the methods (exercise, supplement, and combination) was significant, despite an increase in plasma TAC levels. 4 weeks is likely a short time for changes in plasma TAC levels. On the other hand, there is a possibility that the duration, volume, and rest periods of exercise, and the duration of supplementation have an important effect on the achieved result. It should be noted that the assessment of plasma TAC and MDA response provides only possible information about the oxidative stress caused by exercise.

Our study had several limitations. A limitation of the present study was that our study participants were diabetic patients with age more than 50 years and obese. Given that these participants are at high risk, it was better to take exercise tests from these participants before the study. However, no risk was observed in this study, but future studies can consider this issue. In the present study, superoxide was not evaluated, which could be another limitation of the present study. Diabetic patients use purine nucleotide cycle due to impaired carbohydrate metabolism and the breakdown of inosine monophosphate resulting in the production of superoxide, which should be considered in future studies. Failure to assess blood glucose level, lipid profile, insulin resistance index and hemoglobin A1C are other limitations of the present study that should be considered in future studies.

## Conclusions

The present study shows that HIIT associated with spirulina supplementation are good stimulants to reduce the level of free radical damage and thus reduce risk factors in women with T2DM. Therefore, performing HIIT due to their variety, low-cost time, similar effects to aerobic activities, oxidative damage, and many clinical complications of diabetes, diabetics can benefit from these exercises.

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## Conflict of Interest

The authors declare that there are no conflicts of interest.

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