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# The effect of 12 weeks of Pilates training with Spirulina supplementation on serum Adropin levels in inactive obese women

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**Abstract**

**Introduction:** Adropin is a peptide hormone that plays an important role in energy homeostasis and has recently received attention in regulating adipose tissue metabolism due to its negative association with obesity and insulin resistance. The aim of the present study was to investigate the effect of 12 weeks of Pilates training with Spirulina supplementation on serum Adropin levels in inactive obese women.

**Methods**: The present study was a quasi-experimental and applied study, conducted with a pre-test and post-test design with a control group. In this way, 60 inactive obese women were selected through simple random sampling and divided into four groups of 15 people, including the control group, the Pilates + Spirulina exercise group, the exercise group, and the Spirulina group. The Pilates exercise intervention (65 to 75% of maximum heart rate) was performed for 12 weeks and three sessions per week. Spirulina supplement (in the form of 500 mg capsules) was consumed twice daily after breakfast and evening for 12 weeks. Blood samples were taken before and 48 hours after the last exercise session in a 12-hour fasting state to determine serum Adropin concentration. The data were analyzed using one-way analysis of variance at a significance level of $P<0.05$

**Results**: Intra-group results showed that serum Adropin levels increased significantly in the training ($P=0.0001$), supplement ($P=0.001$) and training + spirulina ($P=0.0002$) groups. Body BMI decreased significantly in the training ($P=0.001$), spirulina supplement ($P=0.001$) and training + spirulina ($P=0.0002$) groups. Significant between-group changes were observed in Adropin ($P=0.0002$), BMI ($P=0.0001$). The combined effect of exercise and spirulina supplementation resulted in a significant increase in Adropin ($P=0.0002$) and a significant decrease in BMI ($P=0.0001$).

**Conclusions:** The combination of Pilates training and Spirulina supplementation appears to be associated with increased serum Adropin levels and decreased body BMI and weight in inactive obese women

**Key Words:** Pilates exercise, Spirulina supplement, Adropin, inactive obese women

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## 1. Introduction

Overweight and obesity are among the greatest challenges facing the World Health Organization, influenced by numerous factors including age, gender, social factors, dietary patterns, and physical activity. Although all these factors affect body weight, the imbalance between energy intake and expenditure is considered one of the most important factors in overweight and obesity (1). Among the important indicators involved in the control and prevention of obesity and overweight are adipokines (2), which also intervene in the regulation of energy metabolism and the complex interactions between adipose tissue and bone (3). Adipokines appear to play an important role in the development of obesity-related diseases and affect the regulation of energy consumption, insulin function, and the occurrence of some metabolic disorders (4). In this regard, with the discovery of new adipokines regulating energy homeostasis, the attention of many researchers has been drawn to studying factors affecting these adipokines. Adropin is a 76-amino acid adipokine expressed in the heart, brain, kidney, liver, pancreas, skeletal muscle, small intestine, and blood vessels (5). Adropin activates the regulation of genes related to fat metabolism through peroxisome proliferator-activated receptor gamma (6). High levels of adropin increase glucose oxidation by activating the pyruvate dehydrogenase complex (7). Adropin can improve metabolism and glucose intolerance in obese and overweight individuals by inhibiting the transfer of free fatty acids into mitochondria (8). It has been reported that low levels of adropin are associated with insulin resistance in humans. Additionally, it has been shown that adropin levels have a negative correlation with age, BMI, and carbohydrate intake, and a positive correlation with fat intake as a percentage of total energy (7). Hepatic expression and circulating concentrations of adropin show rapid regulation through fasting (inhibition) and feeding (stimulation), suggesting regulation by metabolic status signals (9). Soori et al., citing Kumar (2008) who conducted the first animal study on this hormone, reported that the gene encoding adropin in liver tissue is regulated by nutrition. They also observed a decrease in its expression in the liver tissue of rats with diet-induced obesity or genetic obesity (10). In this regard, in the first human study conducted by Butler et al. (2012), the association between adropin levels and insulin resistance in obese individuals was examined, showing that adropin levels have a negative correlation with age, body mass index, and carbohydrate intake (11). Furthermore, the results of studies on adropin indicate that adropin plays a role in metabolic homeostasis and that low serum adropin levels are associated with dyslipidemia (12). It is believed that increased adropin activates the pyruvate dehydrogenase complex to increase glucose oxidation, and in parallel with this event, reduces muscle fatty acid oxidation by inhibiting carnitine palmitoyltransferase-1B, which is the key enzyme for transferring fatty acids to muscle mitochondria for beta-oxidation. Treatment with adropin by inhibiting the transfer of free fatty acids into mitochondria can have therapeutic roles in improving metabolism and glucose intolerance in obesity (8). Therefore, adropin can provide hope for advancing the treatment of metabolic disorders associated with obesity. Nevertheless, despite several years having passed since the discovery of adropin, few studies have examined this peptide.

On the other hand, in recent decades, the use of herbal medicine as a therapeutic approach in controlling obesity disorders has attracted considerable attention (13). Spirulina is one of the herbal supplements with anti-inflammatory properties and contains nutrients such as phycocyanin, chlorophyll, polysaccharides, and sulfolipids that increase energy in the body (14). Research results show that Spirulina is effective in regulating lipid metabolism and prevents the accumulation of hepatic lipids and improves lipid profile (15). Spirulina also contains vitamin B1, which improves sugar metabolism in the body, and vitamin B6, which helps burn calories and prevents obesity (16).

In this regard, Golestani et al. (2021) reported that Spirulina consumption (two 500 mg tablets per day) and HIIT exercises for 4 weeks improved lipid profile in obese women (17). Also, the results of Kheirabadi et al.'s research (2020) showed that Spirulina supplement and HIIT training reduce inflammatory factors resulting from overweight and obesity (18). Lee et al. (2008), after 12 weeks of Spirulina supplementation, reported that this supplement affects blood lipid factors and inflammatory variables (19). According to the researchers, no study has yet investigated the effect of this supplement on the Adropin index. Therefore, due to the lack of sufficient research background in this area, further studies on the duration and effective dosage of this supplement on research indicators are necessary.

Despite the use of various therapeutic approaches in fat reduction, physical activity is among the non-pharmacological and effective therapeutic strategies for treating and preventing overweight and obesity. Physical activity in various forms (based on type, intensity, and volume of training) leads to weight loss, body fat percentage reduction, and related diseases (9). Accordingly, research results showed that physical activities in the experimental group compared to the control group showed a significant decrease in Adropin concentration (9, 20).

Exercise training plays an essential role in weight control by reducing visceral fat mass and reducing the release of pro-inflammatory cytokines and creating an anti-inflammatory environment (21). Aerobic exercise is the best-studied exercise model in interventions related to obesity (22). This exercise model can improve many side effects associated with obesity by using larger muscles (22). Pilates is a set of specialized exercises that engage the body and brain in a way that affects the body's strength, endurance, and flexibility. Unlike most exercise programs that focus only on the physical aspect of the individual, this exercise also focuses on the mental aspect and is based on the harmony between body and mind (23). Kordi et al. (2023) showed that high-intensity interval training significantly increases fibrinolysis in inactive obese women (24). Cheragh Birjandi et al. (2023) in their study showed that Pilates and aerobic exercises caused weight and fat loss in the experimental group compared to the control group (22). Since few studies have been conducted on the effect of Pilates activities and Spirulina supplementation on serum Adropin levels, the present study was conducted to investigate the effect of Spirulina supplementation and Pilates exercises on serum Adropin levels in obese women.

## 2. Methods

*2.1. Participants*

The present study was a quasi-experimental study with a pre-test-post-test design with a control group. The statistical population of the present study included inactive obese women with an age range of 40-45 years in Maragheh city. Initially, an announcement was posted in virtual groups and notices were placed around the city to inform about the objectives and implementation of the research. Volunteers participating in this study were informed about the objectives and implementation method, benefits, and potential risks and entered the study after obtaining informed consent. Inclusion criteria included obesity, body mass index between 30 to 35 kg/m$^2$, age range between 40 to 45 years, no cardiovascular diseases, diabetes, hypertension, orthopedic problems, and medical restrictions preventing participation in activity, no use of

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specific medications, nutritional and medicinal supplements, no smoking and alcohol consumption, no participation in regular physical activity during the past 6 months, no allergy to seafood, and not being menopausal. Exclusion criteria included consecutive absence from three training sessions, injury preventing continued training, and illness (26).

All stages of the research protocol implementation, including informing subjects about the research implementation process, using safe and healthy equipment for research implementation, and keeping their personal information confidential, were implemented. Subjects were randomly divided into four groups: control group, exercise+Spirulina group, exercise group, and Spirulina group, with 15 people in each group. In the simple random method, each subject was assigned a number from 1 to 60, then numbers 1 to 15 were placed in the control group, 16 to 30 in the exercise+Spirulina group, 31 to 45 in the exercise group, and 46 to 60 in the Spirulina group. The control group did not participate in any exercise program during the study and were advised to continue their daily routine as usual. All measurements were performed 48 hours before and after the exercise intervention and Spirulina supplementation. To evaluate body composition, weight (without shoes and with minimal clothing) and height were measured using a SEKA scale and stadiometer (made in Germany) with an accuracy of 0.1 kg and 0.1 mm, respectively, and the waist-to-hip ratio of subjects was measured using a tape measure, and body mass index was calculated by dividing weight (kg) by height squared (meters).

All information is included in the study flow diagram according to CONSORT guidelines.

### *2.2. Study design*

Present the overall experimental design clearly and chronologically. State whether the study was randomized, double-blind, placebo-controlled, crossover, or parallel-group. For crossover designs, report the washout period. Describe the number of experimental conditions or trials (e.g., placebo, low dose, high dose) and the order of administration if applicable. Provide a timeline of procedures including familiarization, run-in period, main trial days, and any follow-up assessments. For each trial day, specify arrival time, fasting status or standardized meal, timing of supplementation, exercise protocol, and post-exercise measurements. If applicable, describe how randomization was performed and how allocation concealment was achieved.

### *2.3. Sample size calculation*

Using G-power software, the minimum sample size was determined to be 60 people, considering an alpha of 5%, test power of 85%, and effect size of 1 (25). 60 of the research samples were grouped based on inclusion criteria using simple random sampling.

### *2.4. Nutritional intervention / Supplementation protocol*

Individuals in the Spirulina supplement and exercise+Spirulina supplement groups consumed 2 capsules of 500 mg Spirulina (manufactured by Rayhan Company) daily in the morning and evening meals at 9 AM and 4 PM, respectively, after meals (29). The capsules for both the exercise+Spirulina supplement group and the Spirulina supplement group were consumed in the same shape and size.

### *2.5. Exercise protocol*

The exercise groups participated in a 12-week Pilates exercise program. While the control group maintained their usual lifestyle during the study period. The Pilates exercise program was performed for 12 weeks, three sessions per week, each session lasting 40 to 60 minutes on even days of the week, including 10 minutes of warm-up, the main body of the exercise consisting of Pilates exercises for 20 to 40 minutes including 12 to 16 movements, and 10 minutes of cool-down. With the progression of the training, time and the number of movements were increased. The intensity of exercises was 65 to 75% of maximum heart rate (controlled by Polar heart rate monitor). At the end of the main exercises, 10 minutes of cool-down (stretching movements) was considered (28).

### *2.8. Statistical analysis*

To check the normal distribution of data, the Shapiro-Wilk test was used, and to check the homogeneity of variances, Levene's test was used. Due to the significance of the above tests, the dependent t-test was used to evaluate within-group differences, and one-way analysis of variance (ANOVA) was used to examine between-group differences. In case of significance, Tukey's post hoc test was used to determine differences between groups. All statistical analyses were performed with SPSS software version 26 at a significance level of P≤0.05.

## 3. Results

### 3.1. Participants' characteristics and compliance

Table 3 shows the mean age (years) and height (meters) of subjects in the control, exercise+Spirulina, exercise, and Spirulina supplement groups. According to the one-way analysis of variance test, research variables between the study groups in the pre-test stage did not show significant differences (P>0.05). The mean and within-group and between-group changes of descriptive indicators of variables before and after the intervention in the four study groups are presented in Table 4 and Figure 1.

Table 1 Pilates Exercise Protocol

**12-Week Pilates Exercise Program**

| Component | Week 1–2 | Week 3–4 | Week 5–6 | Week 7–8 | Week 9–10 | Week 11–12 | Cool-down |
|---|---|---|---|---|---|---|---|
| Warm-up | 10 minutes of stretching exercises and marching in place (each session) | | | | | | 10 minutes of gentle stretching exercises and walking (each session) |
| Duration of main Pilates exercises | 20 minutes | 25 minutes | 30 minutes | 40 minutes | 40 minutes | 40 minutes | |
| Number of exercises | 12 exercises | 12 exercises | 13 exercises | 14 exercises | 15 exercises | 16 exercises | |
| Repetitions | 5 repetitions | 6 repetitions | 7 repetitions | 8 repetitions | 9 repetitions | 10 repetitions | |
| Sets | 2 sets | 2 sets | 3 sets | 3 sets | 3 sets | 3 sets | |
| Intensity | 65–70% | 65–70% | 65–70% | 65–75% | 65–75% | 65–75% | |

Table 2. Pilates Exercises Included in the Training Protocol

| Pilates Exercises Used | |
|---|---|
| Single Leg Circle | Open Leg Rocker |
| Roll Up | Spider Stretch |
| The Hundred | Swan |
| Single Leg Stretch | Side Kick |
| Double Leg Stretch | Double Leg Kick |
| Criss Cross | Spider Dance |
| Double Leg Stretch (Straight) | Push-Up |
| Single Leg Stretch (Scissors) | Kneeling Side Kick |


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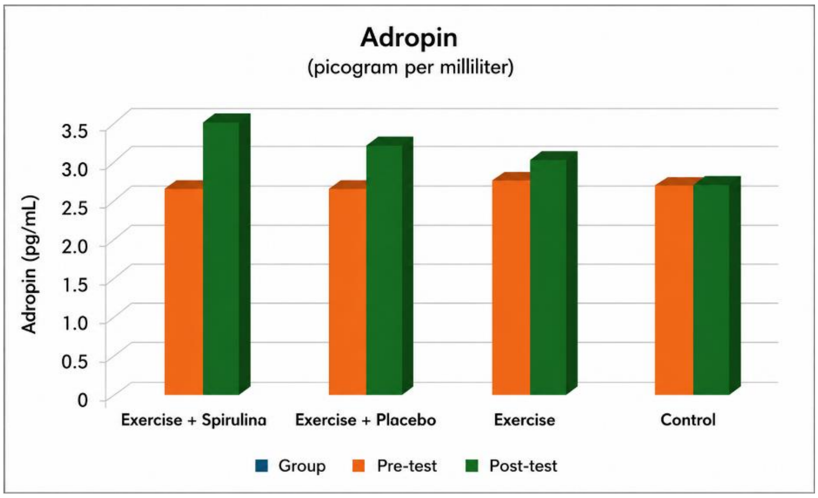
**Table 3.** Mean and Standard Deviation of Age and Height of Participants in Different Study Groups.

| Variables | Control Group | Pilates + Spirulina Group | Pilates Exercise Group | Spirulina Group |
|---|---|---|---|---|
| Age (years) | 42.13 ± 3.05 | 41.97 ± 3.02 | 42.33 ± 4.42 | 41.43 ± 0.08 |
| Height (m) | 1.62 ± 0.05 | 1.61 ± 0.05 | 1.59 ± 0.09 | 1.61 ± 0.07 |

**Table 4. Changes in Study Variables Before and After the Intervention in the Four Study Groups**

| Variable | Time | Control Group | Pilates + Spirulina Group | Pilates Exercise Group | Spirulina Group |
|---|---|---|---|---|---|
| Adropin (pg/mL) | Pre-test | 59.52 ± 2.05 | 48.29 ± 2.00 | 51.42 ± 2.00 | 61.31 ± 2.00 |
| | Post-test | 62.61 ± 2.10 | 49.52 ± 3.22 | 21.41 ± 3.31 | 02.29 ± 3.12 |
| | Within-group P value | 0.023 | 0.001* | 0.001* | 0.001* |
| Body Mass Index ($kg/m^2$) | Pre-test | 31.43 ± 4.33 | 31.52 ± 2.21 | 31.52 ± 3.83 | 31.33 ± 2.36 |
| | Post-test | 30.19 ± 1.31 | 30.11 ± 1.33 | 30.51 ± 0.14 | 30.67 ± 3.38 |
| | Within-group P value | 0.105 | 0.001* | 0.001* | 0.003* |
| Weight (kg) | Pre-test | 92.76 ± 6.00 | 92.43 ± 4.29 | 91.39 ± 5.89 | 92.12 ± 0.49 |
| | Post-test | 91.59 ± 0.64 | 87.65 ± 9.32 | 89.21 ± 1.04 | 89.52 ± 7.47 |
| | Within-group P value | 0.074 | 0.001* | 0.002* | 0.003* |
| Waist-to-Hip Ratio (WHR) | Pre-test | 0.962 ± 0.187 | 0.963 ± 0.544 | 0.965 ± 0.973 | 0.962 ± 0.492 |
| | Post-test | 0.964 ± 0.048 | 0.931 ± 0.298 | 0.943 ± 0.381 | 0.954 ± 0.032 |
| | Within-group P value | 0.109 | 0.001* | 0.001* | 0.001* |

**Figure 1.** Adropin Levels Before and After the Intervention in the Four Studied Groups.



## 4. Discussion

The aim of the present study was to investigate the effect of Spirulina supplementation and Pilates exercises on serum Adropin levels in inactive obese women. The results of this study showed that in the between-group comparison, body weight values had a significant difference. Also, in the within-group comparison, Adropin values in the exercise+Spirulina group, body weight and body mass index in the exercise, Spirulina, and exercise+Spirulina groups, and waist-to-hip ratio in the exercise+Spirulina and exercise groups showed significant decreases. Since no precise study was found on Pilates exercises and Spirulina on Adropin levels, we will discuss similar studies in this field.

The results of the present study showed that 12 weeks of Pilates exercises and Spirulina supplementation increased Adropin in the intervention groups compared to the control group. Several studies have measured Adropin levels in various subjects. Omidi et al. (2019) in their study investigated the effect of a period of low-intensity aerobic exercise on serum Adropin levels in obese adolescent boys and showed that Adropin levels in the experimental group compared to the control group showed a significant increase (30). In other studies, Moradi and Ghahramani (2022) (20) and Kermani et al. (2021) (31) showed an increase in Adropin levels in obese subjects after physical activity, and the results obtained from these studies were consistent with the results of the present study. The results of previous studies indicate the positive effect of physical exercises in increasing Adropin levels; consequently, it is expected that in individuals with chronic diseases including obesity and diabetes, physical activity can have a positive effect on increasing serum Adropin levels in these individuals (30). Although the precise mechanism causing changes in Adropin during physical activity or increased fat metabolism is not yet fully understood (8). Nevertheless, some fundamental and important speculations and probabilities can be mentioned for the relationship between serum Adropin concentration and physical training. It has been reported that weight loss with various interventions such as diet and physical activity improves insulin sensitivity through inflammatory markers or increased mRNA content of glucose transporter protein 4, which in turn increases serum Adropin (32). Also, disruption in glucose and fatty acid metabolism regulation is a metabolic effect in obesity, in which glucose consumption decreases and fatty acids become the primary fuel source in muscles, and limiting excess fatty acids in muscles significantly improves glucose tolerance (33). Increased Adropin activates the pyruvate dehydrogenase complex to increase glucose oxidation, and in parallel with this event, reduces muscle fatty acid oxidation; therefore, Adropin has a therapeutic role in metabolism and glucose intolerance in obesity (8). It appears that the mechanism of Adropin increase in the fasting state and after physical activity is due to glycogen and liver ATP depletion, as well as the role of Adropin in reducing food intake (34).

It is stated that Adropin levels have a negative relationship with age and body mass index (35). The results of 30 minutes of acute exercise in obese subjects showed that despite the effect on weight, serum Adropin levels did not show significant changes (36). On the other hand, considering that Adropin suppresses the differentiation of white adipocytes into mature fat cells and affects lipid cells, stimulating lipolysis and suppressing lipogenesis (27). Consequently, Adropin can reduce body weight by modulating energy consumption and white adipose tissue function and can play an important role as a regulator of fat metabolism (35), and considering the direct relationship with weight and body composition, with the decrease in BMI in the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intervention groups, the influence of these factors on increasing Adropin is more likely. The results of Alizadeh et al. (2018) (36) are inconsistent with the results of the present study, and the reasons for inconsistency can be attributed to the type of exercise, exercise intensity, and type of subjects. It seems that in the present study, Pilates exercises due to sufficient intensity and volume of training stimulated Adropin, and with decreased Adropin, disorders in obesity-related indicators occur in individuals. In this regard, it has been stated that this involves inhibition of the pyruvate dehydrogenase kinase complex followed by increased pyruvate dehydrogenase activity, and it has also been stated that Adropin reduces muscle fatty acid oxidation by inhibiting carnitine palmitoyltransferase-1B, which is the key enzyme for transferring fatty acids to muscle mitochondria for beta-oxidation; therefore, this process can play an important role in obesity (27).

Spirulina supplementation intervention in the present study was effective in increasing serum Adropin levels and improving body composition indicators, and this supplement can prevent oxidative damage caused by free radicals in obese and overweight individuals. The results of this study faced limitations such as the long duration of the training period, and researchers tried to somewhat control the subjects' diet and prevent any regular sports activities other than the training program, but precise control of these factors in human studies is not entirely possible.

## 5. Conclusion

Spirulina supplementation combined with Pilates exercises, by increasing Adropin values and reducing some body composition indicators, can be effective in weight loss and obesity management. It appears that Spirulina combined with physical exercises is more effective than exercise alone or supplement alone in fat metabolism in obese individuals. Diet, exercise intensity and duration, type of subjects, and gender can produce different results. Therefore, it is suggested that in future research, changes in Adropin and body composition indicators in chronic patients with different exercise programs, as well as Spirulina supplementation at higher doses and controlled diet in a residential setting with more supervision over subjects, be studied.

## 6. Highlights

Performing Pilates exercises increases serum Adropin levels in inactive obese individuals.

## Statements

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### Conflicts of Interest

All authors declare that there is no conflict of interest.

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