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Original Article

# Spirulina-fortified salad dressing on body composition and anthropometric indices in hypertensive patients: A tipple-blind randomized placebo-controlled trial

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## *Abstract*

***Background:*** Numerous studies have demonstrated that improving anthropometric indices through a healthy diet and exercise can aid individuals with high blood pressure. Additionally, research suggests that consuming Spirulina, a type of microalga, could positively impact body composition and anthropometric indices. The purpose of this study was to determine the effects of spirulina-fortified salad dressing on anthropometric indices and body composition in patients with hypertension.

***Methods:*** Forty-eight (19 men and 22 women) patients with hypertension randomly received either spirulina-fortified salad dressing containing 2 gr spirulina (SSD, n = 22) or a placebo salad dressing (PSD, n = 19) daily for eight weeks. This study was registered at https://www.irct.ir/ (the Iranian registry of clinical trials identifier: IRCT20200404046940N1).

***Results:*** There was a change in body composition and anthropometric indices from baseline to 8 weeks. We observed that the mean change±SD of waist circumference after the SSD intervention (-2.86±4.02 cm) was significantly different from that of the PSD intervention (0.38±9.47 cm). Although no between-group difference was observed, a statistically significant reduction in body fat-free mass (FFM) occurred in the PSD group after the intervention. Meanwhile, within-group mean change±SD of body fat-free mass (FFM, 1.04±5.39 kg) and body fat mass (FM, -0.73±1.83 kg) was significant only in the SSD group. However, no significance between-group difference was observed in terms of FFM and FM changes.

***Conclusion:*** The present study's findings suggest that consuming spirulina-fortified dressing can improve the status of risk factors associated with hypertension and cardiovascular diseases.

***Keywords:*** Spirulina, Anthropometric indices, Body composition, Hypertension, Cohen's d value.

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Obesity is a public health problem that is rapidly expanding worldwide. According to predictions, approximately 85% and 50% of American adults will meet the criteria of overweight and obesity by 2030, respectively (1). Based on a systematic review, the prevalence of obesity among elderly individuals in Iran is 21.4% (95%CI: 26.6–16.9%) (2). Previous studies have suggested a strong correlation between obesity and the incidence of metabolic syndrome, diabetes mellitus, cancer, coronary heart disease, hypertension, and mental disorders (1). Obesity is commonly categorized into subtypes of generalized obesity and abdominal obesity, considering the criteria of body mass index (BMI) and waist circumference (WC), respectively (2). Obese patients experience adipocyte dysfunction, leading to a spectrum of metabolic complications, including systemic and vascular insulin resistance and sympathetic nervous system dysfunction (3, 4).

 

Furthermore, regional body fat distribution plays a determinant role in metabolic disorders. An increased visceral fat is a risk factor for coronary artery disease, dyslipidemia, hypertension, stroke, and type 2 diabetes (5). Genetic predisposition and exposure to environmental factors, including compounds with epigenetic properties, are involved in obesity development (3, 6). Increasingly, developing countries are adopting western lifestyles; i.e., physical inactivity and increasing intake of calorie-dense and fatty foods with high sodium content) (7). Although several strategies have been suggested to prevent and reduce obesity, they seem to have limited success over time, considering the accelerated expanding rate of this disease.

Dietary supplementation has been strongly suggested to enhance the lifestyle of the human. Previous studies have shown that spirulina (arthrospira platensis) can improve body weight and body fat mass in human populations (8-10). Spirulina is a blue-green alga and is considered one of the most effective nutrients in the 21st century due to its nutrient composition and therapeutic properties (11). This filamentous alga has been used as a rich source of protein and micro-nutrients, which has no considerable side effects even in long-time utilizations. Vitamin $B_{12}$, β-carotenes, tocopherols, iron. phenolic acids, and γ-linolenic acid are the most frequent bioactive compounds in spirulina (12). While spirulina supplementation appears to be a promising option for managing obesity, the current body of scientific evidence supporting its efficacy is relatively limited and inconsistent (13).

Miczke et al. assessed the effect of a 3-month intake of 2 grams of spirulina maxima and found that it improved weight (14). In a study conducted by Yousefi et al., daily intake of 2 grams of spirulina platensis for 12 weeks significantly reduced body weight, waist circumference, body fat, and BMI in the SP group compared to the placebo group (15). In contrast, Szulinska et al. and Hernández-Lepe et al. reported that spirulina did not positively impact other anthropometric measurements (16, 17). Based on our comprehensive searches in scientific databases, our study is the first to assess the effect of incorporating spirulina algae into a food product on anthropometric indices. Previous research has primarily focused on spirulina used as a supplement. This clinical trial, therefore, represents a novel approach by evaluating the potential health benefits of spirulina when integrated into everyday dietary items, providing new insights into its applicability and efficacy in a more practical, food-based context. In the present study, we aimed to investigate the effect of spirulina-fortified dressing on body weight and body composition in hypertensive patients.

## Methods

**Study protocol and ethical issues:** Patients provided their written informed consent, and the study protocol was approved by the institute's committee on human research. We assured patients that all of their personal information would remain confidential. The protocol of this study was approved by the Ethics Committee of the Shiraz University of Medical Sciences (Registration Number: 1398.1193). It was also registered at the Iranian Registry of Clinical Trials (Register Number: IRCT20200404046940N1)

**Patients:** In this study, participants were recruited from patients with hypertension referred to Professor Kojuri Cardiology – Super Specialty Clinic between January 2020 and March 2020. Individuals were included in the study if they were afflicted with the stage-I or II of hypertension based on 2017 ACC/AHA guidelines (i.e., SBP ≥ 130 mmHg, DBP ≥ 80 mmHg or both of them), aged between 24 and 65 years, were not suffering from cardiovascular, diabetes, kidney, liver, inflammatory bowel diseases, cancer, did not have a history of cardiac surgery, and were not using any dietary supplement or specific diet plan. Participants were excluded from the study if they did not consume more than 20% of the products or were reluctant to continue. The detailed information on the method and participants' properties has been previously published (18, 19).

**Study design:** This study was a randomized triple-blind placebo-controlled trial. Participants were randomly allocated into two groups: Spirulina salad dressing (SSD), receiving a sachet of salad dressing formulated with 2 grams of spirulina platensis, and the placebo salad dressing (PSD), receiving a resemble sachet formulated with the same sensory properties daily for eight weeks. Reversible blocks were used for the randomization process, and both the researchers and the subjects were kept blind throughout the entire process until the final data were examined. Distinct codes for each group were used to blind the statistician to the data. The prescribed salad dressings were restricted in calories, sugar, fat, and salt. The participants were recommended to consume the products with fresh vegetables. An isocaloric diet containing ~55% carbohydrate, ~15% protein, and ~30% fat was tailored for participants based on their calorie demand calculated by the Mifflin equation (1). The recruited individuals were recommended not to change their usual lifestyle, including physical activity. In this study, the participants, researchers, and statisticians were blinded until the end of the data analysis.

**Anthropometric indices and questionnaire data:** Bodyweight and body composition were measured using

Tanita body composition analyzer (BC 418 MA) while the participants were barefooted and were wearing minimal clothes. The standing height was measured using a standard stadiometer (Seca, Germany, Model No. 222) to the nearest 0.1 cm, and the waist circumference (WC) was measured using a non-stretchable tape with an accuracy of 0.5 cm at the level of the iliac crest and the end of a normal expiration. Body mass index (BMI, kg/m$^2$) was calculated by dividing weight (kg) by squared height (m$^2$) (1). To assess the dietary intake, we collected 24-hour recall for 3 days at the beginning and the end of the trial. These recalls were analyzed by the modified version of Nutritionist IV software (1995, First Databank, San Bruno, California, USA) to estimate the daily intake of macronutrients and micronutrients. The International Physical Activity Questionnaire (IPAQ) was used to evaluate the daily energy expenditure of participants (MET-min/wk) using the metabolic equivalents attributed to different tasks (20). We also used the Beck Anxiety Inventory (BAI) (21) and the Holmes – Rahe stress scale (22) to assess the level of anxiety and stress, respectively. The Pittsburgh Cold Study-3 (PCS3) questionnaire was used to determine participants' socioeconomic status (23).

**Statistical analysis:** The sample size was calculated considering 80% power and 0.05 α-error (10). The Shapiro-Wilk test was applied to examine the normality of data distribution. Baseline between-group comparisons of continuous variables were performed by the independent samples t-test or non-parametric test of the Mann-Whitney. Meanwhile, the paired samples t-test or the Wilcoxon signed-rank test was applied to analyze the within-group comparisons. The chi-square test was used to investigate the differences in frequencies of categorized variables. Moreover, the value of Cohen's d (Cd) was calculated to assess the magnitude of the effect sizes.

Based on the definition, Cd is the between-group difference of post-interventional mean changes divided by the pooled standard deviation (SD). For the Cd values of 0.20, 0.5, and 0.80, the magnitude of effect sizes were considered "medium", "small", and "large", respectively. A Cd = 0 means no considerable between-group difference in effect sizes. In other words, a Cd value of zero indicates that 50% of observations in the control group are lower than the average value of the intervention group. Also, the average value in the intervention group locates at the percentile of 58$^{th}$, 69$^{th}$, and 79$^{th}$ of the distribution curve of observations in the control group if the Cohen's d value is 0.20, 0.50, and 0.80, respectively (24). All statistical analyses were performed using the SPSS software Version 21 (Inc. Chicago, IL).

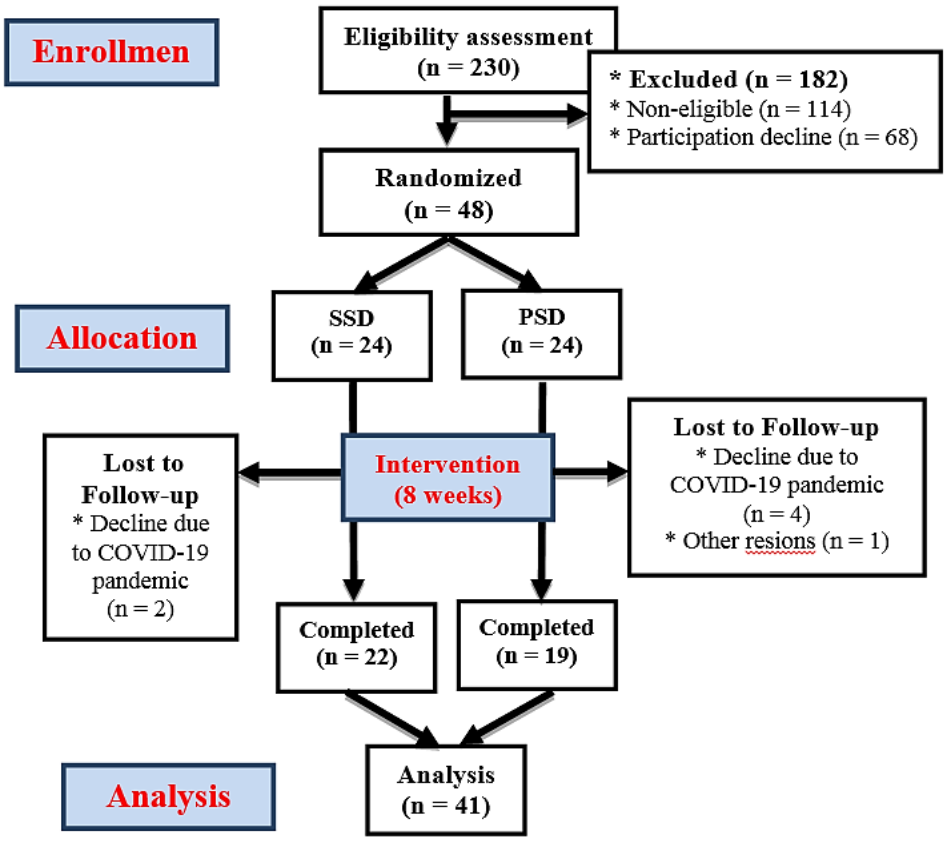


**Figure 1. Flow diagram of the study**
(SSD: Spirulina-fortified salad dressing, PSD: Placebo salad dressing)

## Results

As shown in the flow diagram of the study (figure 1), 7 out of the total 48 initially randomized participants were excluded due to different reasons, and the final analyses were conducted with 22 participants in the SSD group and 19 in the PSD group (n=41, Male=19, Female=22). According to the findings, no significant between-group difference was observed for baseline characteristics of age, gender, smoking, education degree, anti-hypertensive medication therapy, and physical activity level (table 1). Also, baseline comparisons showed no significant difference between the two groups in terms of quantitative variables, including age, Daily Energy Expenditure (DEE), and dietary intake, except for polyunsaturated fatty acids and cholesterol (table 2). We found that waist circumference (WC) was significantly reduced only in the SSD group, while a significant reduction in body fat-free mass (FFM) was observed in the PSD group (table 3). However, no statistically significant between-group changes were observed in all parameters of interest (Figure 2). Cohen's d value was less than 0.2 for body weight (BW) and BMI. Meanwhile, the absolute value of this statistic was greater than 0.4 for other outcomes of interest, including WC, FFM, and FM (figure 3). We also assessed the impact of the intervention in body mass index subgroups and discovered that the results remained mostly consistent in these subgroups.

**Table 1. Baseline characteristics of participants for qualitative outcomes**

| Variables [a] | | Overall (n=41) | Treatment group | | Baseline P-value [b] |
|---|---|---|---|---|---|
| | | | *SSD (n=22)* | *PSD (n=19)* | |
| **Gender** | Male | 19 (46.3) | 11 (50) | 8 (42.11) | 0.61 |
| | Female | 22 (53.7) | 11 (50) | 11 (57.89) | |
| **Marital Status** | Single | 2 (4.9) | 0 (0) | 2 (10.5) | 0.40* |
| | Married | 36 (87.8) | 20 (90.9) | 16 (84.2) | |
| | Divorced or widow | 3 (7.3) | 2 (9.1) | 1 (5.3) | |
| **Smoking** | Yes | 7 (17.1) | 3 (13.6) | 4 (21.1) | 0.68* |
| | No | 34 (82.9) | 19 (86.4) | 15 (78.9) | |
| **BMI** | Underweight | 1 (2.5) | 0 (0) | 1 (2.5) | 0.43* |
| | Normal weight | 7 (17.5) | 3 (13.6) | 4 (22.2) | |
| | Overweight or obese | 32 (80) | 19 (86.4) | 13 (72.2) | |
| **Education Degree** | Diploma, or bellow | 19 (46.4) | 8 (36.3) | 11 (57.9) | 0.18* |
| | Associate or Bachelor's degree | 17 (41.5) | 12 (54.6) | 5 (26.4) | |
| | Master's degree or higher | 5 (12.2) | 2 (9) | 3 (15.8) | |
| **Physical Activity** | Low | 15 (36.6) | 7 (31.8) | 8 (42.1) | 0.74* |
| | Moderate | 10 (24.4) | 6 (27.3) | 4 (21.1) | |
| | High | 9 (22.0) | 6 (27.3) | 3 (15.7) | |
| | Very high | 7 (17.1) | 3 (13.6) | 4 (21.1) | |
| **Socio-economic Status** | Low | 4 (9.75) | 3 (13.64) | 1 (5.26) | 0.68 |
| | Middle | 27 (65.85) | 14 (63.63) | 13 (68.42) | |
| | High | 10 (24.4) | 5 (22.73) | 5 (26.32) | |
| **Anxiety Level** | Low | 1 (2.44) | 0 (0.0%) | 1 (5.3%) | 0.05 |
| | Moderate | 18 (43.9) | 13 (59.1%) | 5 (26.3%) | |
| | High | 22 (53.66) | 9 (40.9%) | 13 (98.4%) | |
| **Stress Level** | Low | 17 (41.46) | 9 (40.9%) | 8 (42.1%) | 1.00 |
| | Moderate | 17 (41.46) | 9(40.9%) | 8 (42.1%) | |
| | High | 7 (17.08) | 4 (18.2%) | 3 (15.8%) | |

SSD: Spirulina-fortified salad dressing, PSD: Placebo salad dressing. [a] Data is presented in n (%), [b] Chi-square Q-test or Fisher's exact test (*) was used for between-group comparisons.

**Table 2. Baseline characteristics of participants for quantitative outcomes**

| Variable [a] | | Overall (n = 41) | Treatment group | | Baseline P-value [b] |
|---|---|---|---|---|---|
| | | | SSD (n = 22) | PSD (n = 19) | |
| **Age (year)** | | 50.78±5.99 | 51.27±6.12 | 50.21±5.95 | 0.54* |
| **DEE (kcal/day)** | | 2396.73±288.93 | 2390.29±283.58 | 2404.59±304.41 | 0.88 |
| **Blood Pressure** | SBP (mmHg) | 142.81±15.21 | 144.72±13.99 | 140.60±16.63 | 0.39 |
| | DBP (mmHg) | 93.98±12.49 | 92.59±10.39 | 89.70±13.01 | 0.43 |
| **Daily Dietary Intakes** | Energy (kcal) | 1592.0±537.4 | 1609.06±639.70 | 1571.40±404.42 | 0.69 |
| | Carb (gr) | 266.50±90.63 | 271.35±101.26 | 260.95±78.91 | 1.00 |
| | Protein (gr) | 59.34±27.04 | 60.76±31.36 | 57.69±21.73 | 0.81 |
| | Fat (gr) | 39.00±14.60 | 36.59±17.63 | 41.37±9.84 | 0.07 |
| | Fiber (gr) | 30.00±14.50 | 28.58±12.97 | 30.86±16.44 | 0.81 |
| | SFA (gr) | 12.70±7.00 | 13.12±8.68 | 12.16±4.47 | 0.79 |
| | PUFA (gr) | 8.30±4.34 | 6.91±3.77 | 9.85±4.52 | 0.04 |
| | MUFA (gr) | 12.40±4.70 | 11.3±5.06 | 13.66±3.96 | 0.06 |
| | Cholesterol (mg) | 266.50±90.63 | 214.43±136.2 | 189.36±189.22 | 0.04 |

SSD: Spirulina-fortified Salad Dressing, PSD: Placebo Salad Dressing, DEE: Daily Energy Expenditure, WC: Weight circumference, BMI: Body Mass Index, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, Carb: Carbohydrate, SFA: Saturated Fatty Acids, MUFA: Mono Unsaturated Fatty Acids, PUFA: Poly-Unsaturated Fatty Acids, Na: Sodium, K: Potassium, Ca: Calcium, Mg: Magnesium. [a] Data are presented in mean ± SD, [b] Independent Sample t-test or Mann-Whitney U-test (*) was used for between-group comparisons.

**Table 3. Anthropometric indices before and after the spirulina and placebo interventions**

| Variables [a] | Treatment group | | | | | |
|---|---|---|---|---|---|---|
| | SSD (n=22) | | | PSD (n=19) | | |
| | Before | After | *P-value* [b] | Before | After | *P-value* [b] |
| **BW (kg)** | 83.14±2.76 | 82.57±2.81 | 0.21 | 81.82±5.01 | 79.41±5.63 | 0.05 |
| **WC (cm)** | 102.65±1.61 | 99.80±1.74 | 0.00** | 102.47±3.82 | 102.85±3.08 | 0.86 |
| **BMI (kg/m$^2$)** | 29.75±0.98 | 29.55±2.81 | 0.24 | 29.78±1.61 | 29.44±1.53 | 0.08 |
| **FFM (kg)** | 54.00±2.15 | 55.06±2.06 | 0.37 | 54.09±3.36 | 52.80±3.45 | 0.04 |
| **FM (kg)** | 28.25±2.01 | 27.51±2.203 | 0.07 | 26.69±3.62 | 28.93±3.41 | 0.18 |

SSD: Spirulina-fortified Salad Dressing, PSD: Placebo Salad Dressing, BW: Bodyweight, WC: Waist circumference, BMI: Body Mass Index, FFM: Fat-Free Mass, FM: Fat Mass

[a] Data is reported in mean ± SD.

[b] Mean difference of variables was the average of within-individual differences calculated by subtracting initial values from the final values.

[c] Paired sample t-test or Wilcoxon signed-rank test (*) was used for within-individual comparisons.

[d] Independent sample t-test or Mann-Whitney U-test (*) was used for between-group comparisons.

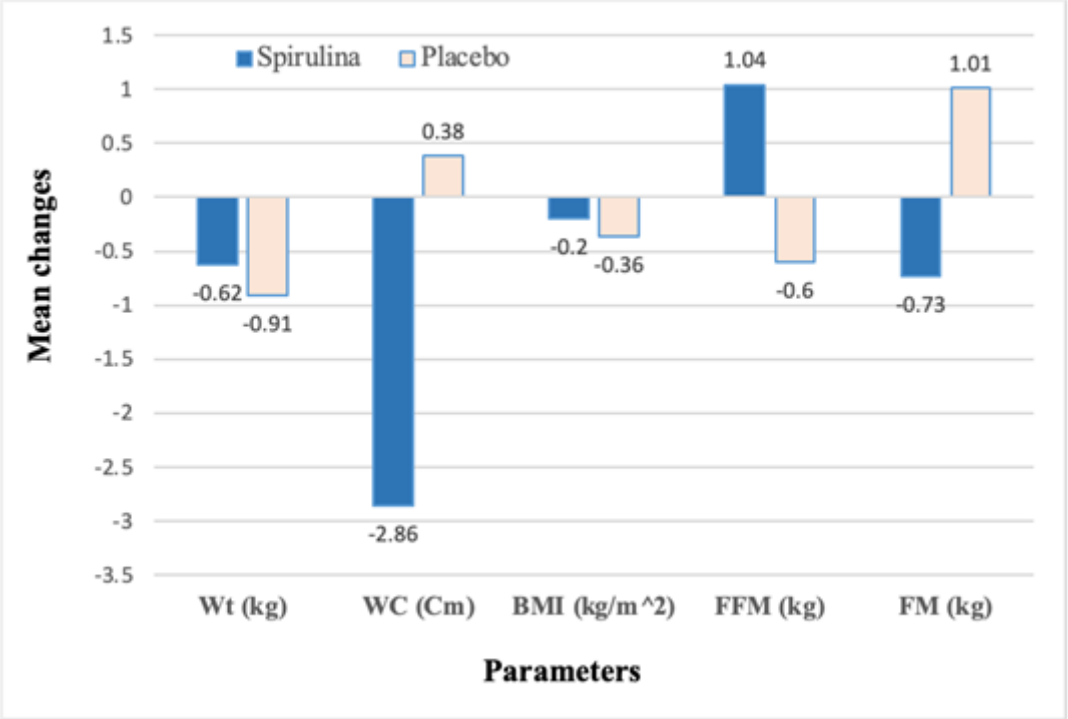


**Figure 2. Mean changes of anthropometric response variables in the spirulina and placebo groups**
Wt: Body Weight, WC: Waist circumference, BMI: Body mass index, FFM: Fat free mass, FM: Fat Mass

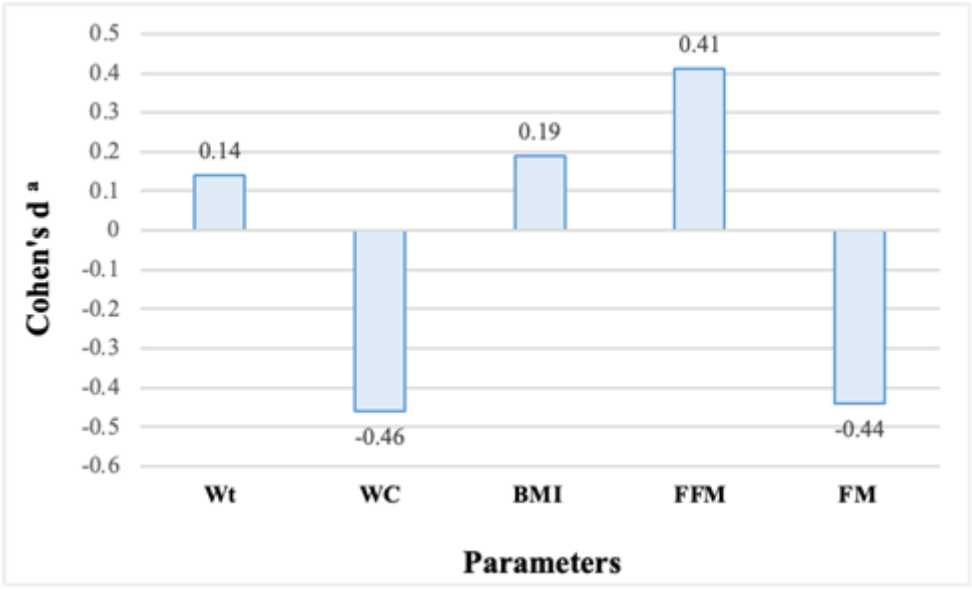


**Figure 3. Effect size estimation overall changes of anthropometric indices at the end of the study.**
[a] Cohen's d value was defined as the post-interventional mean difference of parameters, divided by the pooled standard deviation. Wt: Bodyweight, WC: Waist circumference, BMI: Body Mass Index, FFM: Fat Free Mass, FM: Fat Mass

## Discussion

According to this study, waist circumference was significantly reduced in the spirulina group. This finding is of great clinical importance because regional body fat distribution plays a determinant role in metabolic disorders. An increased visceral fat is a risk factor for coronary artery disease, dyslipidemia, hypertension, stroke, and type 2 diabetes (5). It is shown that insulin sensitivity is lower in patients with higher central fat deposition leading to higher concentrations of circulating insulin and increased turnover and concentration of non-esterified fatty acids (25). In the state of obesity, the reduced responsiveness of visceral

adipocytes to insulin results in increased fatty acid delivery to the liver (26). Also, the activity of hepatic lipase and lipoprotein lipase may be affected by the altered concentration of circulating insulin (27, 28). Reduced lipoprotein lipase activity decreases the maturation of HDL cholesterol, while the elevated activity of hepatic lipase increases HDL cholesterol catabolism. All of these events can lead to atherosclerosis and micro-vascular disorders.

The findings of this clinical trial were consistent with some previous studies. A clinical trial study of middle-aged dyslipidemia patients with overweight or obesity found that daily consumption of 1 gram of spirulina for 12 weeks did not alter body weight and BMI. The researchers declared that the ineffectiveness could be due to low doses of spirulina used for treatment (29). However, another study in South Korea examined the effectiveness of higher doses of spirulina (8 g/day for 12 weeks) on anthropometric indices. Similarly, this intervention did not cause significant changes in body weight and body mass index of patients with diabetes. It is noteworthy that the average BMI of the participants in the above study was about 23.50, which indicates the normal body weight of the study population (30). Nonetheless, we also evaluated the effects of the intervention in the subgroups related to body mass index and found that the findings remained almost unchanged in BMI subgroups.

However, some previous studies indicate a significant However, some previous studies indicate a significant effect of spirulina consumption on anthropometric indices. One study observed that spirulina supplementation (1 g/d for 12 weeks) could effectively reduce body weight, BMI, and appetite (9). It has also been reported that overweight patients with hypertension experienced a significant reduction in BMI and weight by 3-months of consumption of 2 g/day spirulina (10). In another study, patients with non-alcoholic fatty liver disease (NAFLD) who consumed 6 g/d of spirulina for 6 months experienced a significant decline in weight (31). These inconsistencies led us to seek meta-analyses to draw clearer conclusions on this matter. A recent meta-analysis of 5 clinical trials showed that spirulina consumption could significantly reduce body weight, waist circumference, and body fat mass (13). However, the results of a sensitivity analysis in another meta-analysis study revealed that the use of spirulina in studies with an intervention period of more than 12 weeks led to a significant reduction in body mass index (32). So far, several mechanisms have been suggested for the effect of spirulina on anthropometric measurements. One of the well-known mechanisms is that spirulina can reduce the infiltration of macrophages into visceral fat and inhibit liver lipid accumulation and oxidative stress. Moreover, spirulina can act as an appetite suppressant due to its considerable content of phenylalanine, which is a potent releaser of cholecystokinin, reducing body weight and visceral fats (29, 33). It is also reported that the antioxidant content of spirulina would contribute to obesity treatment through various mechanisms such as suppression of food intake, stimulation of energy expenditure, adipocyte differentiation, and regulatory effect on lipid metabolism, including lipase inhibition (34). In the present study, we observed that WC and FFM were reduced in the spirulina group in the opposite direction to FM. Meanwhile, the value of Cohen's d was greater than 0.4 for these three variables indicating a significant treatment-induced clinical alteration. The coincidence of these changes with non-significant alterations in body weight in the two groups could support the claim that the fat mass in the waist area may have been replaced by fat-free mass in the participants of the spirulina group.

The present study had some limitations. We used salad dressing as the carrier of spirulina (2 gr/sachet/day) in this study. The intervention dose in this study was not considered further primarily due to the significant portion of oil in the salad dressing formulation. Increasing the intervention dose could significantly affect the participants' dietary intake of fatty acids and energy. Another limitation of the present study was its short intervention period. Two highly-influential factors can affect epidemiological studies in our country: the arrival of the holy month of Ramadan (Moslems fasting month) and the Nowruz holiday (Persian New Year). The lifestyle of people in these two periods is quite different from their usual style. In the present study, to curb the effect of this confounding condition, we designed the study schedule so that salad dressing production would begin immediately after the holy month of Ramadan and the entire intervention finish before the Nowruz holiday. On the other hand, spreading Covid-19 disease was our further limitation in this study. This made some participants reluctant to continue and complete the study. To counteract the consequent sample loss, we conducted final measurements for these individuals by visiting their homes in compliance with health protocols. However, some participants declined further cooperation and were subsequently excluded from the study.

In the present study, we found that daily consumption of a salad dressing formulated with 2 gr of spirulina reduced waist circumference and body fat mass and increased lean body mass. These findings are of great importance since it is shown that body fat mass, especially in the central region, is directly and strongly associated with the incidence of

many metabolic diseases. However, to provide a more comprehensive understanding of the issue, it is necessary to conduct additional well-designed clinical trials with larger sample size and longer duration in the future.

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**Conflict of interests:** The authors have no conflicts of interest to declare.

**Authors' contribution:** Ghaem Far Z, Babajafari S and Mazloomi SM participated in propose the original idea of the study, supervise the work. Ghaem Far E and Ghaem Far Z also carried out the stages of intervention, data collection, draft the article, and review. Mazloomi SM also contributed to formulation of the products, investigation of required analytical tests, quality as well as the safety of the products. Nouri N, Mohammadi S and Rahmani MH contributed to draft and revision of the article and final approval of the manuscript. Ashrafi-Dehkordi contribute to data analysis, statistical analysis, and interpretation. Kojuri J visited the patients and carry out the stages of intervention. All authors read and approved the final version of the manuscript.

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