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Spirulina Platensis effectively ameliorates anthropometric measurements and obesity-related metabolic disorders in obese or overweight healthy individuals: A randomized controlled trial

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Highlights

- Anthropometric measures including body weight, waist circumference, body fat and BMI significantly reduced following daily intake of 2 gr *spirulina platensis* concurrently with calorie restricted diet for 12 weeks.
- Serum triglyceride level better reduced in the *spirulina* receiving group compared to the placebo group.
- Spirulina supplementation improved high sensitivity C-reactive protein level compared to the placebo group.
- Appetite score was significantly reduced in the SP group compared to the baseline

Abstract

Aims: Novel alternative treatments such as food supplements may be an effective approach to weight management. The aim of the present study was to investigate the possible effects of *Spirulina Platensis* (SP) on anthropometric measures, appetite and metabolic parameters in obese or overweight individuals.

Material and methods: A total of fifty-two obese and overweight subjects (25 kg/m^2 ≤body mass index (BMI)<40 kg/m^2) were randomly selected to be allocated to SP (4×500 mg daily tablets along with restricted calorie diet (RCD)) or placebo (placebo tablets along with RCD) for 12 weeks of intervention. Anthropometric measurements and appetite score were assessed at baseline, weeks 6 and 12. Biochemical assessments were performed at baseline and week 12.

Results: Thirty-eight participants completed the intervention. Body weight, waist circumference, body fat and BMI significantly reduced in the SP group compared to the placebo

group ($p<0.001$, $p=0.049$, $p=0.049$ and $p=0.02$, respectively). In the SP group, the reduction triglycerides (TG) and high sensitivity C-reactive protein levels was considerably significant compared to the placebo group ($p= 0.03$, $p= 0.02$, respectively). Appetite score was significantly reduced in the SP group compared to the baseline ($p<0.001$).

Conclusions: This study suggests that *spirulina platensis*, as a complementary therapy may have beneficial effects on adherence to RCD, management of weight loss and also reduction in TG levels through possible modulatory effects on anti-inflammatory pathways.

Key words: *spirulina platensis*, Obesity, body mass index, triglyceride, *hs*-CRP

Trial registration: ClinicalTrials.gov Identifier: NCT02993627; Registered December 15, 2016

1. Introduction

Obesity as one of the most important worldwide public health issues [1, 2], is associated with a wide range of metabolic disorders such as chronic inflammation, dyslipidemia [3], and impaired glucose tolerance [4]. Cardiovascular diseases, type 2 diabetes, and other related metabolic abnormalities are also the dire consequences[4, 5]. Therefore, achieving the best weight management strategy is an important component of public health and wellbeing. So far, restricted calorie diet (RCD) has been considered as the first line of therapy for weight management. As adherence to RCD for losing weight is difficult, Iranians like other eastern populations are eager to use nutraceuticals or herbal medications for accelerating the weight loss process [6]. In such context, it seems reasonable to find the appropriate complementary therapy

along with RCD for maintaining the better results in weight management and obesity-related disorders. *Spirulina Platensis* (SP), blue-green microalgae with spiral filaments[7], is regarded as a functional food due to its high nutritional components and potential therapeutic values[8, 9]. A few clinical trials have been evaluated the effects of SP on anthropometric measurements, body composition and the associated metabolic disorders, and also the findings seem to be rather uncertain[10-12]. *Spirulina's* medical and nutritional efficacy maybe attributed to its nutrient content; a rich protein source (approximately 60–70% of dry weight) with high protein quality, containing almost all essential amino acids[7]. SP is rich in antioxidants like phycocyanins [7-9] making it a suitable supplement for lowering blood lipids [7] and inflammation [13]. Based on powerful antioxidant compounds of spirulina, it is proposed as a successful supplement for lowering oxidative stress and inflammation [13], mostly through scavenging free radicals and reactive oxygen species (ROS), suppressing the activity of factor KB (NF-kB) and decreasing the pro-inflammatory cytokines production [13-15]. Furthermore, adipose tissue as an active metabolic and endocrine organ secretes several endocrine substances such as adipokines which may also have important effects on inflammatory status or lipid metabolism, especially in obesity status[16]. Therefore, SP as an alternative therapy may play a key role in inflammation or dyslipidemia management in individuals with obesity. On the other hand, based on the current knowledge, there is a general lack of research investigating the effects of SP on obesity management along with diet therapy, inflammatory diseases, dyslipidemia and underlying mechanisms [10, 17, 18].

Consequently, the present study aimed to compare the potential effects of a 2 g/day SP supplementation along with RCD concurrently with RCD alone for 12-week on anthropometric indices, serum lipids, appetite, *hs*-CRP and adiponectin levels in adults with overweight or obesity.

2. Methods and design

2.1. Design

This study is a randomized, double-blinded, placebo-controlled clinical trial. The recruitment was conducted at the Nutrition and Diet Therapy Clinic of Shahid Beheshti Medical University, Tehran, Iran. The subjects were enrolled in the study through public announcements at a local public center, notifications to the students and staff of the Department of Nutrition and the patients attending the clinic. According to Szullnska *et al*, [19] a difference of 5.1 kg/m^2 unit in BMI was expected following the intervention with a power (1-β) of 95% (α=0.05). The required sample size was calculated to be at least 20 subjects in each group. Considering the possible dropout rate, 52 obese and overweight men and women (25 kg/m^2 ≤BMI<40 kg/m^2), aged between 20 and 60 years were recruited for this study. The study duration was defined according to similar studies performed previously [12]. According to the previous investigations, daily intake of 2 gr SP was considered to be the appropriate dosage to reach the potential benefits of supplementation[12]. The primary outcomes include a significant improvement in weight, BMI, appetite, body composition, waist and hip girth. Secondary outcomes included serum *hs*-CRP, adiponectin, blood lipid concentrations. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and The study protocol was approved by the Ethics Committee of the National Nutrition and Food Technology Research Institute of Iran (reference no: IR.SBMU.nnftri.Rec.1395.63). Written informed consent was obtained from all subjects, after the protocol and aim of the study were completely explained to them. This clinical trial was registered on the ClinicalTrials.gov (identifier: NCT02993627) where full trial protocol can be accessed. This study was conducted between December 2016 and December 2017 by the

Department of Nutrition and Food Technology of the Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2.2. Patients

Inclusion criteria included: to be categorized as overweight or obese (25 kg/m^2≤BMI<40 kg/m^2), aged between 20 and 60. Exclusion criteria included: any history of cardiovascular, chronic kidney, autoimmune and infectious diseases, hypo or hyperthyroidism, more than 3 kilogram weight change in the past two months, recent surgery, dieting or under a controlled dietary regimen over the previous six months, receiving some type of drug therapy or complementary and alternative medicines that lowered the body weight, lipid profile, or blood glucose, taking vitamins/minerals, functional food or antioxidant supplements, pregnancy or lactation.

2.3. Randomization

A trained dietician performed randomized permuted block stratification as follows; the individuals were stratified according to body mass index (BMI) (three levels: 25 kg/m^2 ≤ BMI< 30 kg/m^2, 30 kg/m^2 ≤ BMI< 35 kg/m^2, and 35 kg/m^2 ≤ BMI< 40 kg/m^2) and randomly allocated to either the SP or the placebo group by block randomization[20]. This block randomization was conducted by a block size of 4 and possible balanced combinations with 2 C (control) and 2 S (SP) subjects, as 6 different ways could have been performed: CSCS, CGSS, CSSC, SCCS, SCSC and SSCC. Then, blocks were randomly chosen, using a simple random sampling method to determine the assignment of all the participants into the groups. Furthermore, all the procedures, such as generation of the random allocation sequence, enrolling the participants, and assigning these participants to interventions, were performed by one person.

2.4. Intervention

The participants of the SP group received four tablets of SP every day (two tablets in the morning and two tablets in the evening, after meals) along with RCD, whereas the individuals in the control group received four placebo tablets daily, in addition to RCD. The supplements were comprised of 500 mg of SP and placebo tablets contained starch and lactose monohydrate. The placebo and SP tablets had an almost identical appearance. The participants were asked to maintain their usual level of physical activity along with their prescribed diet during the intervention period. The participants of both the SP and placebo groups received low-calorie diet (-500 kcal) composed of ~55% carbohydrate, ~15% protein, and ~30% fat during the study period [21] which were prescribed by a qualified dietician. Adherence to the study protocol was maintained with the help of regular telephone calls and constant contact on cyberspace (Telegram messenger). The participants were requested to bring the remaining tablets back and the pill count back method was applied to assay the adherence rate. The compliance rate was calculated as higher than 90% percent.

2.5. Preparation of SP supplement

Spirulina platensis tablets (Spiruvit®) are manufactured by Ashbal Chemi Co. LTD, at Shahre Daru Lab Co. Spiruvit Active Pharmaceutical Ingredient (API) is supplied in developed laboratories of Roquette Klötze GmbH & Co. KG, Germany in full standard conditions and it is free of any additives. Roquette uses the new technology of Photobioreactor in order to make the Spiruvit powder. By this method, microalgae are grown in closed system and controlled conditions. Therefore, the contaminants will be omitted and the final product will have the premium quality. The nutrient facts of the prescribed supplements in this survey included as

follow: 290 kcal, 7.73gr fat, 23.94gr carbohydrate, 57.47gr protein, minerals 4.54gr, vitamins 0.52gr, and 5gr water in 100 gr *Spirulina platensis*.

2.6. Anthropometric assessment

Body weight was measured with the minimum clothing and without shoes, with a precision of 100g (Seca, CA, USA). The patients were requested to remove their shoes for measuring their height and this was done using a wall-mounted stadiometer at baseline. Afterwards, their BMI was calculated, weight (kg)/ height^2 (m^2). The waist and hip circumferences were measured to the nearest 0.1 cm using an inflexible tape at the smallest circumference below the rib cage and above the umbilicus , and at the largest circumference between the waist and knees [22], respectively. A bioelectric impedance analysis device (X-Contact 356 Body Composition Analyzer, United Kingdom) was used to assess lean body mass, total fat, and muscle mass at baseline, Weeks 6, and Week 12 of the study.

2.7. Biochemical assessments

After 12-hr overnight fast, 10 ml of venous blood was collected into serum-separating tubes from all the participants at baseline and at the end of intervention. The blood samples were centrifuged at 3500 rpm for 20 min and the serum samples were separated and stored at −80ºC. Biochemical analysis of total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein (HDL-C), triglyceride (TG) was done following standard procedures recommended on the Pars Azmoon diagnostic kits (Tehran, Iran). A human adiponectin ELISA kit (ZellBio GmbH, Germany) with an intra-assay *CV* of less than 10% was employed to detect the adiponectin level. The serum *hs*-CRP level was also measured using a commercial ELISA kit

(DBC, Canada) with an intra-assay *CV* of 9.5%. The sensitivity of the kits for adiponectin and *hs*-CRP were 0.1mg/L and 10ng/mL, respectively.

2.8. Physical activity level, appetite and dietary assessment

Physical activity level was assessed using a valid questionnaire and calculating metabolic equivalent (MET) at baseline, Week 6, and Week 12 of the study[23]. The appetites of the subjects at the beginning, at Week 6, and at the end of the intervention, were assessed using a simple appetite questionnaire (score ranged from 4 to 20)[24]. A 24-hr diet recall (for two week days and one weekend) was used to assess the dietary intake of the participants at Weeks 0, 6, and 12 of the intervention. The dietary intake data were analyzed using the Nutritionist IV software (N Squared Computing, San Bruno, CA, USA).

2.9. Statistical Analysis

Statistical analysis was performed using the SPSS software (IBM Corp., released 2012, IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp|). Intention-to-treat principle (ITT) and per-protocol analysis were applied for analyzing the data; the per-protocol analysis results were only displayed because of the same outputs. A Kolmogorov-Smirnov test of normality was used to verify the distribution of variables. The variables are reported as mean ± standard deviation, separately. Natural log transformations were used for assessment of normality, which were performed on the serum TG level and fiber intake. Furthermore, the serum adiponectin level was transformed with the help of the Box-Cox transformation. For comparing qualitative variables between the two groups, a χ^2 test was used. Repeated measures ANOVA and paired *t*-test were employed to compare parameters within groups. For comparing parameters between groups, independent sample *t*-test was used and in order to adjust for

confounding factors (changes in body weight, waist circumference, body fat, BMI, energy intake, age, sex and baseline physical activity), an analysis of covariance was also performed. The assumption of homogeneity of variance was tested using Levene's test. A p-value of ≤ 0.05 was considered as statistically significant.

3. Results

Fifty-two individuals were determined to be eligible for the study and were categorized randomly to receive SP or placebo. Among the participants, 38 people successfully completed the trial and were enlisted in the final analysis. Two participants from the SP group and three from the placebo group were excluded due to non-compliance (consuming less than 90% of their supplements). One of participants from the SP group complained of intestinal discomfort, one underwent medical treatment for an unrelated condition, and another became pregnant. Hence, these three participants had to withdraw from the study. Moreover, four subjects in the placebo group and two in the SP group were lost to follow-up due to miscellaneous reasons (Figure 1).

3.1. Baseline characteristics

An analysis of the baseline characteristics is represented in Table 1. There were no significant differences between treatment groups in terms of age, sex, smoking, and past experiences with weight reducing treatment.

3.2. Dietary intakes and physical activity level

Dietary intake of total energy, macronutrient, and micronutrient were not statistically significant between the two groups at baseline, Weeks 6 and 12 (Tables 2). Energy, protein, and carbohydrate intakes were significantly reduced in both group as compared to baseline ($p<0.001$). Furthermore, dietary intake of saturated fatty acid (SFA) was significantly reduced in

the SP group compared to baseline (p=0.01) and fiber intake was significantly decreased in the SP and the placebo group in comparison to the baseline (p=0.01, p<0.001; respectively). The physical activity level was considerably higher in the SP group at baseline (p = 0.04). However, the participants in the placebo group increased their level of physical activity notably during the study period (p<0.001). However, changes in the physical activity level were not significantly different between the two groups at Week 6 or Week 12. All the variables were adjusted for age and sex.

3.3. Effects on anthropometric measurements and appetite

The anthropometric parameters and appetite values are represented in Table 3. Body weight, BMI and lean body mass (LBM) were significantly reduced in the SP group compared to the placebo group (p<0.001, p=0.01 and p=0.04; respectively). Waist circumference was also significantly reduced in the SP group (p=0.049) at the end of the study. Furthermore, BF reduction was significantly higher in the SP group than in the placebo group (3.37 ± 2.65 cm *vs.* 1.73 ± 2.37 cm; p=0.049) at week 12. Reduction in body weight, BF, % BF, waist circumference and appetite was significantly higher in the two groups in comparison to the baseline (p<0.001 and p=0.001, p<0.001 and p=0.001, p<0.001 and p=0.03, p<0.001 and p=0.001, p<0.001 and p=0.04; in the SP and the control groups, respectively). Moreover, in the SP group, BMI, hip circumference and WHR significantly reduced in comparison to the baseline (p<0.001). Appetite score reduced significantly in the SP group compared to the baseline (p<0.001). Also, Anthropometric measurements and appetite were adjusted for sex, age, energy intake, and baseline physical activity.

3.4. Effects on biochemical assessments

The baseline concentrations of biochemical parameters were not different between the two groups (Table 4). The *hs*-CRP level in the SP group was significantly lower in comparison to the placebo group at Week 12 (p= 0.03). Moreover, the TG and *hs*-CRP levels reduced significantly after 12 weeks of intervention in the SP group as compared to the placebo group (p= 0.03 and p=0.01, respectively). Also, a substantial decline was observed in TG, LDL-C, LDL-C/HDL-C ratio, and hs-CRP following the 12 weeks of SP supplementation compared to baseline (*p*= 0.02, <0.001, 0.04 and 0.04, respectively). The biochemical biomarkers were adjusted for age, sex, weight, waist circumference, BMI, body fat, baseline physical activity and energy intake.

4. Discussion

This trial investigated the effects of SP concurrently with a RCD on obesity management and obesity-related metabolic disorders in adults with obesity and overweight. This functional food improved anthropometric measurements including weight, waist circumference, body fat and BMI along with *hs*-CRP and TG level compared to the placebo group.

Based on scientific literature, the relationships between SP and obesity indices are controversial. Our results seem to corroborate the finding of Miczke *et al*, [12] who reports that a 2 g/day *Spirulina maxima* supplementation for three months could decrease the weight and BMI of hypertensive and overweight patients. Also, Zeinalian *et al*, [10] showed that the consumption of 500 mg SP twice a day for three months reduced the weight and BMI in obese individuals. To the best of our knowledge, this is the first study that evaluated the anti-obesity and metabolic consequences of SP as a complementary therapy along with RCD. We demonstrated that weight reduction (-3.22 kg in the SP group *vs* -1.45 kg in the placebo group) in the SP group based on the calorie intake reduction during the study (-381 in the SP group kcal *vs* -369 kcal in the placebo group) was significantly higher than the placebo group. Furthermore, BMI, waist

circumference and body fat was also significantly reduced in the SP group compared to the placebo group at the end of the study. In contrast, some studies revealed that spirulina supplementation for 12 weeks had no influence on anthropometric parameters[8, 17]. Although, these conflicting findings may be related to the metabolically healthy or unhealthy participants, supplement dosage and the duration of the study. The underlying mechanism of anti-obesity effects of SP has been rarely discussed, but it seems that the proposed mechanisms involved are reduction of macrophage infiltrations into visceral fat, preventing liver-lipid accumulation, ameliorating oxidative stress [25] and reducing appetite [26].

As obesity is considered a low-grade inflammation state which is associated with the development of obesity-related comorbidities [5, 27]; anti-inflammatory nutritional interventions have recently been proposed to manage this pro-inflammatory status [28]. The omega-3 fatty acids and polyphenols are among the principal components of anti-inflammatory dietary patterns [28], which is also the main components of SP [7, 13]. Furthermore, Phycocyanin and ß-carotene seem to be the most effective anti-inflammatory and antioxidant components of SP[29, 30], which may be responsible for the beneficial effect of SP on inflammatory status. Previous studies also recommended SP as a nutraceutical food for inflammation management through plasma interleukin-2 (IL-2), adiponectin levels, and plasma concentration of tumor necrosis factor- α (TNF-α) [8, 11, 31]. In this study, *hs*-CRP level significantly decreased by 26.8% in the SP group compared to the baseline and 8.6% higher level of adiponectin was also observed in the SP group in comparison to the baseline which was not significant. This modulatory effect of our intervention on *hs*-CRP could be attributed to the anti-inflammatory properties of SP components or higher weight loss in the intervention group. Although body weight adjustment through ANCOVA test for *hs*-CRP level suggested that ameliorating the role of SP in *hs*-CRP level

management probably was not under the influence of body weight or BMI. Another proposed mechanism is appetite controlling effect of SP [10], in the present study appetite score decreased significantly in the SP group compared to the baseline, however that was not significantly different in comparison to the placebo group. It is suggested that phenylalanine content of spirulina may be responsible for cholecystokinin release which is effects the appetite center of brain [17]. However, further studies are required for clarifying the appetite modifying effect of SP.

Another important aspect which was assessed in this study was obesity-related dyslipidemia. Dyslipidemia is generally characterized by hypertriglyceridemia or hypercholesterolemia and lower level of HDL-C[32]. The present research results are in good agreement with the previous reports which shows the hypolipidemic effects of SP, specially through TG and total cholesterol reduction and increasing level of HDL-c [8, 12, 33]. Our results showed significantly lower levels of TG in the SP group (-18 mg/dL *vs* 6mg/dL) compared to the placebo group. Furthermore, reducing level of LDL-C and LDL-C/HDL-C compared to the baseline was only observed in the SP group. Disturbances in TG and HDL-C levels seems to be correlated with pro-inflammatory cytokines, which is caused by the infiltration of macrophage into visceral adipose tissue[34]. Therefore, improvement of the TG level in the SP group in the present study may be somehow related to inflammation management and lower macrophage infiltration into adipose tissue. This TG reduction could also be explained through weight reduction in this group[35], however weight loss adjustment through ANCOVA did not change the result. Another possible mechanisms which should be addressed is the increase in activity level of lipoprotein lipase, hepatic triglyceride lipase [36] and inhibition of intestinal cholesterol and bile acid absorption [17].

Despite promising results, there are some limitations such as small sample size with the higher female participants, not evaluating the different component of SP individually and not assessing

the level of other inflammatory biomarkers such as IL-6 and TNF-α. In addition, it is important to consider that our subjects are metabolically healthy obese subjects so our results may not be generalizable to the unhealthy obese population. In spite of these limitations, to our knowledge, this is the first study to show that anti-inflammatory effects of SP can have a favorable effects on weight management through double blinded randomized design.

The results of this dietary intervention study highlights that RCD concurrently with a 2 g/day SP supplementation for 12 weeks, compared to RCD alone, ameliorated anthropometric measurements, inflammatory status, and serum TG levels in obese or overweight subjects. These results suggests that *spirulina platensis* as a functional food supplement may play a fundamental role in individual adherence to RCD and possibly efficient weight management in obesity and dyslipidemia through anti-inflammatory mechanisms. Further clinical trials are also required to generalize these findings to the entire population of obese individuals and also conduct more comprehensive assessment of inflammatory biomarkers.

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Conflict of interest statement

None of the authors had any financial or personal conflicts of interest.

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Authors' Contributions

Authors' Contributions to the manuscript were as follows: R.Y. designed and conducted research, provided essential materials, performed statistical analysis, wrote paper, and had primary responsibility for final content; A.S. designed research, performed statistical analysis, wrote paper, and had primary responsibility for final content; A.M. designed research, and had primary responsibility for final content. All authors read and approved the final manuscript to be published.

Conflict of interest

The authors declare that they have no conflict of interest.

Declarations of interest: none.

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Table 1. Baseline characteristics of the subjects in SP and placebo groups *

Characteristics	SP (n=19)	Control (n=19)
Age (years)	40.16±10.8	39.79±8.26
Sex (n/%)		
Male	6 (31.6%)	1 (5.3%)
Female	13 (68.4%)	18 (94.7%)

Past experiences with weight-reducing treatment (n/%)		
Yes	15 (78.9%)	13(68.4%)
No	4 (21.1%)	6 (31.6%)
Smoking (n/%)		
Yes	1 (5.3%)	1 (5.3%)
No	18 (94.7%)	18 (94.7%)

*Values are means ± SDs
SP: Spirulina Platensis

Table 2. Dietary intakes and physical activity in SP and placebo groups [1]

Variables	Study period				
	Baseline	Week 6	Week 12	Changes during 6 weeks	changes during 12 weeks
Energy (g/d)					
SP	1918±675	1643±6396	1536.42±479*	-275.05±127	-381.81±90
Placebo	2016±566	1616±444	1646.88±408*	-399.75±124	-369.19±101
Protein (g/d)					
SP	72.99±25.99	64.2±23.88	57.75±18.28*	-8.79±5	-15.25±4
Placebo	79.74±18.59	67.58±21.82	64.97±16.67*	-12.16±4	-14.77±4
Carbohydrate (g/d)					
SP	267.86±121.85	197.65±94.37	196.73±83.14*	-70.2±19	-71±18
Placebo	257.37±82.9	192.18±67.23	205.95±65.81*	-65.2±16	-51.4±15
Fat(g/d)					
SP	65.05±24.98	67.9±26.43	59.32±16.59	2.86±8	-5.72±4
Placebo	76.74±28.08	65.69±13.73	64.72±14.03	-11.04±6	-12.02±4
SFA (g/d)					
SP	19.57±6.71	16.46±5.86	16.02±4.34**	-3.11±1.2	-3.54±1.4
Placebo	16.6±6.79	15.85±7.45	14.36±4.23	-0.75±1	-2.24±1.4
MUFA (g/d)					

Variables	Study period				
	Baseline	Week 6	Week 12	Changes during 6 weeks	changes during 12 weeks
SP	18.33±7.65	20.48±8.2	19.37±8.1	2.15±2	1.04±2.1
Placebo	22.67±8.59	19.19±5.95	21.87±9.03	-3.47±2.4	-0.79±2
PUFA (g/d)					
SP	25.7±14.26	22.93±6.88	19.88±6.47	-2.77±3.2	-5.82±3
Placebo	20.15±14.41	23.64±12.59	19.6±10.51	3.48±4.3	-0.55±2.7
Cholesterol (mg/d)					
SP	255.71±133.15	223.83±118.54	246.68±71.27	-31.89±25	-9.03±32
Placebo	236.77±129.45	234.74±127.11	189±118.77	-2.03±37	-47.76±28
Fiber(g/d) [§]					
SP	15.37(10.07, 20.67)	11.8(10.26, 13.21)	13.68(10.9, 16.36)**	-3.52(-9.59, 0.00)	-2.85(-7.85, 1.52)
Placebo	15.68(10.25,20.3)	10.76(9.38,12.58)	10.9(.3, 13.67)*	-2.44(-8.75, 0.59)	-3.67(-7.19, 0.08)
Physical activity level (MET/day)					
SP	36.3±3.65†	36.38±3.71	36.47±3.6	0.08±1.33	0.17±1.08
Placebo	34.07±2.71	34.54±2.77	34.92±2.81*	0.46±1.22	0.85±1.17

[1] Values are means ± SDs

* *p<.001* versus baseline

** p<.05 versus baseline

† *p<.05* versus the placebo group. Analysis of covariance for changes during 12 weeks (adjusted for age, sex, baseline value of physical activity)

[§] Values are presented as medians plus range

SP: Spirulina Platensis; MUFA: monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; SFA: Saturated fatty acid

Table 3. Anthropometric parameters, body composition and appetite in SP and placebo groups*

Variables	Study period			changes during 6 weeks	changes during 12 weeks
	Baseline	Week 6	Week 12		
Body weight (Kg)					
SP	86.85±10.65	85.32±10.51	83.63±10.61 †	-1.53±2.17	-3.22±1.97 §
Placebo	85.55±13.12	84.63±13.37	84.1±13.39 †	-0.92±1.49	-1.45±1.86
BMI (kg/m^2)					
SP	32.67±4.49	32.06±4.07	30.85±5.35 †	-0.62±0.85	-1.23±0.79 ‖
Placebo	32.99±4.29	32.63±4.35	32.42±4.32	-0.37±0.59	-0.63±0.68
BF (kg)					
SP	32.73±7.21	31.71±6.54	30.45±6.69 †	-1.02±1.64	-2.28±1.74
Placebo	33.57±7.21	33.33±7.47	32.36±7.33 †	-0.24±1.32	-1.22±1.55
BF (%)					
SP	37.58±6.17	37.28±5.72	36.37±5.79 †	-0.29±1.24	-1.21±1.17
Placebo	39.12±3.41	39.17±3.48	38.32±3.79 ‡	0.04±1.14	-0.8±1.51
LBM (kg)					
SP	54.15±8.2	51.17±13.29	53.08±8.14 †	-2.98±9.35	-1.07±1.12 ‖
Placebo	51.75±6.93	50.98±6.64	51.62±7.5	-0.77±0.97	-0.13±1.59
Waist (cm)					
SP	103.16±9.21	102.1±8.52	99.79±8.81 †	-1.05±2.17	-3.37±2.65
Placebo	101.1±9. 4	99.57±9.89	99.36±9.32 †	3.21±21.18	-1.73±2.37
Hip (cm)					
SP	118.47±9.16	117.58±9.02	116.63±9.61 †	-0.89±1.52	-1.84±2.06
Placebo	119.68±8.86	118.95±8.62	118.53±8. 4	-6±22.87	-1.16±2.95
WHR					
SP	0.87±0.07	0.87±0.07	0.86±0.07 †	-0.002±0.01	-0.01±0.02
Placebo	0.84±0.05	0.84±0.05	0.84±0.05	-0.007±0.02	-0.006±0.02
Appetite score					
SP	15.42±1.98	14.42±1.98	14.21±1.27 †	-1±1.53	-1.21±1.36
Placebo	15±2.38	14.63±1.67	14.26±1.59	-0.37±1.57	-0.74±1.52

* All values are mean ± SD
† $p<.001$ versus baseline
‡ $p<.05$ versus baseline
§ $p<.001$ versus the placebo group, Analysis of covariance for changes during 12 weeks (adjusted for age, sex and energy intake)
‖ $p<.05$ versus the placebo group, Analysis of covariance for changes during 12 weeks (adjusted for age, sex and energy intake)
SP: Spirulina Platensis; BF: body fat, BMI: body mass index, LBM: lean body mass, WHR: waist to hip ratio

Table 4. Plasma concentrations of biochemical parameters in the SP and control groups

Biochemical variables		Study period		Changes during 12 weeks
		Baseline	Week 12	
TG (mg/dL) §				
	SP	110(87,205)	94(117,141) *	-18 (-77,-2) ‡
	Placebo	91(80,170)	104(84,184)	6(-5,20)
TC (mg/dL)				
SP		198.79±42.82	191.42±41.6	-7.37±21.42
	Placebo	183.73±40.68	186.05±39.08	2.31±22.95
LDL-C (mg/dL)				
SP		114.58±25.57	105.53±24.84 †	-9.05±12.53
	Placebo	102.79±24.78	100.05±21.13	-2.73±15.4
HDL-C (mg/dL)				
SP		52.42±9.44	51.47±11.32	-0.95±7.08
	Placebo	50.79±9.15	50.1±5.93	-0.68±6.08
LDL-C/HDL-C				
SP		2.22±0.47	2.07±0.37*	-0.15±0.29
	Placebo	2.03±0.38	2±0.38	-0.03±0.18
Adiponectin (mg/L) §				
SP		10.4(8.8,28)	13.6(9.1,44.2)	0.9(-1.4,5.7)
Placebo		9.8(8.7,14.8)	11.9(8.6,21.2)	-0.1(-0.9,4.4)
***hs*-CRP (ng/mL)**				
SP		6.18±2.9	5.09±3.94* ‡	-1.66±1.9 ‡
Placebo		5.38±2.4	6.93±3.7	1.54±4.8

* $p<.05$ versus baseline, † $p<.001$ versus baseline, ‡ $p<.05$ versus the placebo group, †† $p<.001$ versus the placebo group

§ Values are presented as medians Q1–Q3, 25th–75th percentile

Analysis of covariance for changes during 12 weeks (adjusted for baseline values and also age, sex, body fat, body weight, BMI changes and energy intake) & independent sample t-test for variables

SP: Spirulina Platensis; HDL-C: high density lipoprotein- cholesterol; *hs*- CRP: high-sensitivity C-reactive protein; LDL-C: low density lipoprotein-cholesterol; TC: total cholesterol, TG: triglyceride

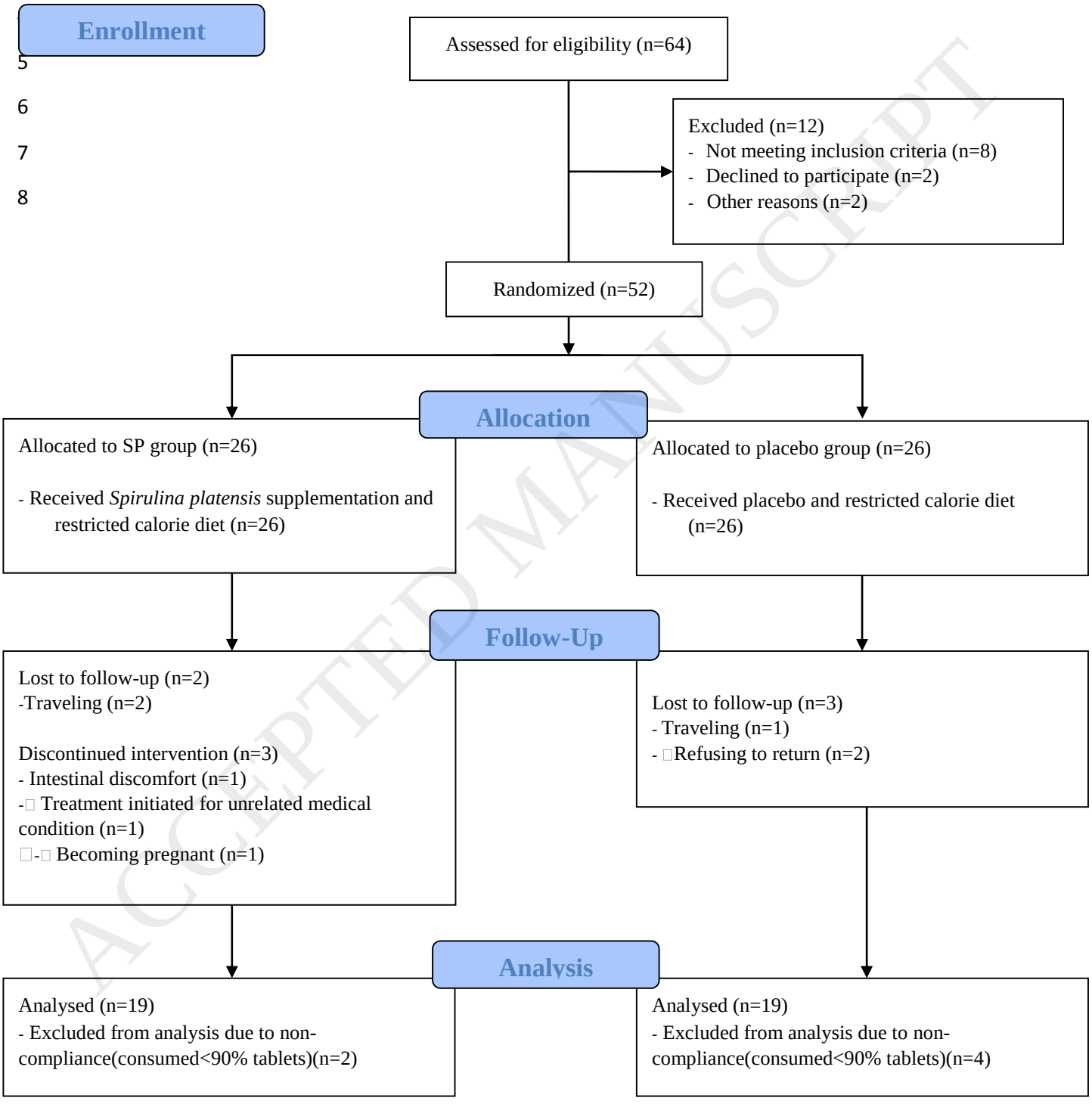

Fig. 1. The study protocol.