Original Article

Spirulina for Protection Against COVID-19 via Regulating ACE2, FNDC5, and NLRP3: A Triple-Blind Randomized Placebo-Controlled Trial in Obese Adults

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Abstract

Background: Spirulina may protect individuals against viral infections and promote health in obese subjects. This study is designed to investigate the impacts of spirulina on obesity to find a hope to protect this population against COVID-19.

Materials and Methods: In a double-blinded randomized placebo-controlled trial, 24 obese subjects (Mean age: 44.83±3.04 years; mean weight: 111.95±22.55kg; body mass index (BMI): 40.31±6.03kg/^{m2}) were randomly allocated to spirulina (n=12) or control (Co, n=12) groups. Spirulina was administered 2 gr/day for 8 weeks and the Co group received a placebo for a similar period. Before and after the administration of spirulina, the anthropometric measurements were calculated for each subject. Furthermore, ACE2, NLRP3, and FNDC5 gene expression were examined in adults with obesity.

Results: Our findings demonstrated that spirulina could not effective in normalizing body weight (BW), BMI, and waist-hip ratio (WHR). Spirulina administration significantly upregulated the gene expression of FNDC5 and significantly reduced NLRP3 and ACE2 gene expression in obese subjects compared with the Co-group. Furthermore, by increasing FNDC5 the gene expression of NLRP3 and ACE2 was significantly reduced.

Conclusion: While administration of spirulina for eight weeks could not affect the anthropometric measurements, it showed the greatest impact on the gene expression of NLRP3, ACE2, and FNDC5, emplacing its potential in the protection of obese cases against COVID-19.

Keywords: Spirulina, Obesity, COVID-19, Inflammasome, Irisin, ACE2

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Introduction

In the past decades, obesity is the most common metabolic disease that influences people throughout the world (1). It has been predicted that in 2030, 20% of worldwide adults will be obese and another 38% will be overweight (2). Obesity is accompanied by clinically important complications, e.g., metabolic

syndrome, heart disease, type 2 diabetes mellitus, hepatic disease, cancer, and pulmonary complications (3). The adipose tissue accumulation and the secretion of various cytokines by adipocytes are associated with several complications (4). Previously, it has been shown that pre-existing conditions such as diabetes, obesity, hypertension, and cardiovascular diseases (CVDs) are related to severe symptoms in subjects with coronavirus disease 2019 (COVID-19) (5). Obesity has been considered a predictive factor in the current COVID-19 pandemic (6, 7). Furthermore, angiotensin-converting enzyme 2 (ACE2) acts as a severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) functional receptor to enter host cells and can be found in adipose tissue. The visceral tissue contains more ACE2 compared to the peripheral subcutaneous adipose tissue (8). ACE2 is also overexpressed in adipocytes and adipocyte-like lipofibroblasts in the lung, which may have an essential role in the pathophysiology of COVID-19 (9). Additionally, ACE2 is an essential part of the renin-angiotensin-aldosterone system (RAAS), which contributes to the regulation of body homeostasis, blood pressure, salt balance, inflammatory responses, and oxidative stress, through angiotensin pathways (10). Imbalanced RAAS system was reported in obese individuals (11).

The NLR family pyrin domain-containing protein 3 (NLRP3) inflammasome can be overexpressed in adipose tissue (12). Besides, COVID-19 is accompanied by activation of innate immune immunity due to the assembly of the NLRP3 inflammasome, leading to caspase-1-mediated production of pro-inflammatory cytokines, including IL-1β and IL-18, and induction of pyroptosis, an caspase 1-dependent cell death (13). Therefore, COVID-19 worsens the preexisting inflammation in obese patients and enhances the risk of hospitalization in this population (14). Furthermore, irisin, a cleavage product of the extracellular domain of fibronectin type III domain containing 5 (FNDC5), can be richly observed in the skeletal muscle (15). The health benefits of irisin in obesity are linked to the 'browning' of white adipose tissue (16). FNDC5 exhibits potential beneficial impacts on COVID-19 consequences via inhibition of the inflammatory response and obesity (17).

Obesity has been reported to be linked to severe forms of COVID-19. So, it is more important than ever to investigate relationships to find strategies to promote national health in this population. Among several herbal supplements, Arthrospira or spirulina shows numerous health benefits such as antitumor, antioxidant, anti-inflammatory, metalloprotective, antiviral, and antibacterial properties (18). Spirulina can promote immunity and exhibit antiviral effects against various enveloped several viral infections, for example, human immunodeficiency virus (HIV) (19). Furthermore, spirulina was effective in the prevention of obesity and obesity-associated complications (20).

In a clinical trial, we aimed to examine the impact of spirulina on obese individuals to normalize their body composition to regulate some genes, including ACE2, NLRP3, and FNDC5, to promote health and protect this population against SARS-CoV-2 infection.

Methods

Study Subjects

This study was an 8-week double-blinded randomized-controlled trial (IRCT20220105053631N1) registered 01 January 2022, https://www.irct.ir/trial/61202). In this study, 24 people (12 men and 12 women) with obese (mean age: 45.16±3.13 yrs.; mean weight: 112.38±20.1 kg, mean BMI: 39.66±6.07 kg/m2). The individuals who received 2 doses of the COVID-19 vaccine were selected through advertisements (social media platforms, posters, short message service **(**SMS), and email). The inclusion criteria included: age of 40- 50 years, BMI>30 Kg/m^2, non-smokers, no history of chronic disorders (e.g., hypertension, CVDs, and type 2 diabetes), no therapy with hormones or psychiatric medications, and no alcohol drinking, and not regular physical activity in the last two months. The subjects consuming dietary supplements and drugs that influence adipose tissue metabolism and muscle mass (such as corticosteroids, beta-blockers, amino acids, beta-agonists, etc.) were excluded. Before the initiation of the investigation, all steps of study and testing were defined for the subjects. Then subjects were requested to sign an informed consent form. The study was performed following the Helsinki Declaration

(Nathanson, 2013) and accepted by the "Ethics Committee of the Islamic Azad University" (Ethics code: IR.IAU.TNB.REC.1400.102).

Study Groups: The subjects were assigned randomly groups to one of two groups: Spirulina or Control (Co). Data gathering was done at two time points; 1) 48 h before starting the investigation and 2) 48h after the last time receiving the spirulina or placebo. The data collection was done at the same time under the same standard conditions (~50-55% humidity and ~20°C heat). During the investigation, the subjects in each group were requested to continue their usual diet. All subjects were living in the same facility. Blinding (participants, researchers, and outcome assessors) with a placebo was used to reduce the likelihood of differential treatment of outcomes. Placebo and spirulina supplements were placed in identical capsules. The daily dosage of spirulina (Qeshm Gulfstar, Iran) was 2.1 g/day and the capsules (700 g each) were taken three times/day after breakfast and before lunch and dinner meals.

Sample size and randomization: The sample size was calculated to find significant differences among research variables with a power value of ≥80% and a confidence interval (CI) of 95% and for the sample size calculation, G*Power software (University of Trier, Trier, Germany) was used according to on generic moderate effect sizes (Cohen's f=0.5), error of 0.05. The sampling method in this study was classified as randomization. The number of cases was people (12 men and 12 women). Since the expression of the effect of the interventionists may have a significant difference according to the gender of the people, therefore, an equal number of each gender was studied in the sampling. Based on this, the sample space was divided into two blocks of 12 people. An online software, a sealed envelope, was used for the randomization; available at https://www.sealedenvelope.com.

Anthropometric indices: The anthropometric indices of subjects including body weight (BW), body height (BH), body mass index (BMI), body fat percentage (BFP), and waist-to-hip ratio (WHR) were measured. Subjects were in light clothing and without shoes during measurements. A calibrated weighing scale was utilized to measure BW (~0.1 kg). BH (~0.1 cm) was determined with a stadiometer. BMI was determined by dividing BW (Kg) by the BH square (m^2). The BFP of subjects was calculated using the Body Composition device (Medigate Company Inc.). To calculate WHR, hip (HC) and waist circumference (WC) were measured. WC was defined as the narrowest diameter between the iliac crest and the lower ribs. HC was assessed around the widest portion of the buttocks using a stiff measuring tape (21). All measurements were performed in duplicate and the final mean values were reported by an investigator.

Blood Sampling: Blood sampling was done 2 days before and 2 days after the study. All the subjects were referred to the laboratory for blood sampling. Fasting blood samples (6 mL) were taken from the antecubital vein of each participant. Serum was prepared and frozen at $-80^{\circ C}$ for quantitative PCR (qPCR),

RNA Extraction and qPCR: The ACE2, NLRP3, and FNDC5 gene expression were measured in the blood samples of obese subjects. The RNX-Plus kit was used for the extraction of total RNA. The obtained solution contained total RNA and its amounts were quantified by a NanoDrop (Thermo Fisher Scientific). For cDNA synthesis from isolated total RNA, a PrimeScript™ RT Master Mix Kit (Takara) was used. For this purpose, 3 µg of isolated RNA along with 0.8 µL of primers were brought to a volume of 12 µL in a microtube. The microtube was mixed, incubated for 5 min, and transferred to ice. 5x reaction buffer, RNase inhibitor, dNTP mix, and Reverse Transcriptase were mixed with the samples. the total volume was brought to 20 µL. The microtubes were mixed well and then, were microfuged. Then they were transferred to the thermocycler and incubated for 1 h at 42°C. PCR Master kit was used for the amplification of PCR products with specific primers. Briefly, 1 µL of synthesized cDNA, 1 µL of forward and reverse primers were prepared, and 1.5 µL of Master Mix were placed in PCR microtubes and brought to a volume of 25 µL with sterile deionized water. The microtubes were mixed well and amplified in a thermocycler. Beta-2 microglobulin (B2M) was considered the internal control gene in this study. Relative mRNA

expression was measured using the 2(ΔΔCt) method.

Dietary Analysis: Food records were done in three days (one weekend day and twice a week) before and after the study were obtained to determine the alterations of the usual diet over time (21). Furthermore, every food item was individually entered into Nutritionist software (Nutritionist IV, Version. 3.5.2) and the amount of energy from fats, carbohydrates, and proteins, as well as total energy consumption, were calculated (Table 2).

Statistical Analysis: data analysis was done by a blind assessor using PSS software (Version 12, IBM®

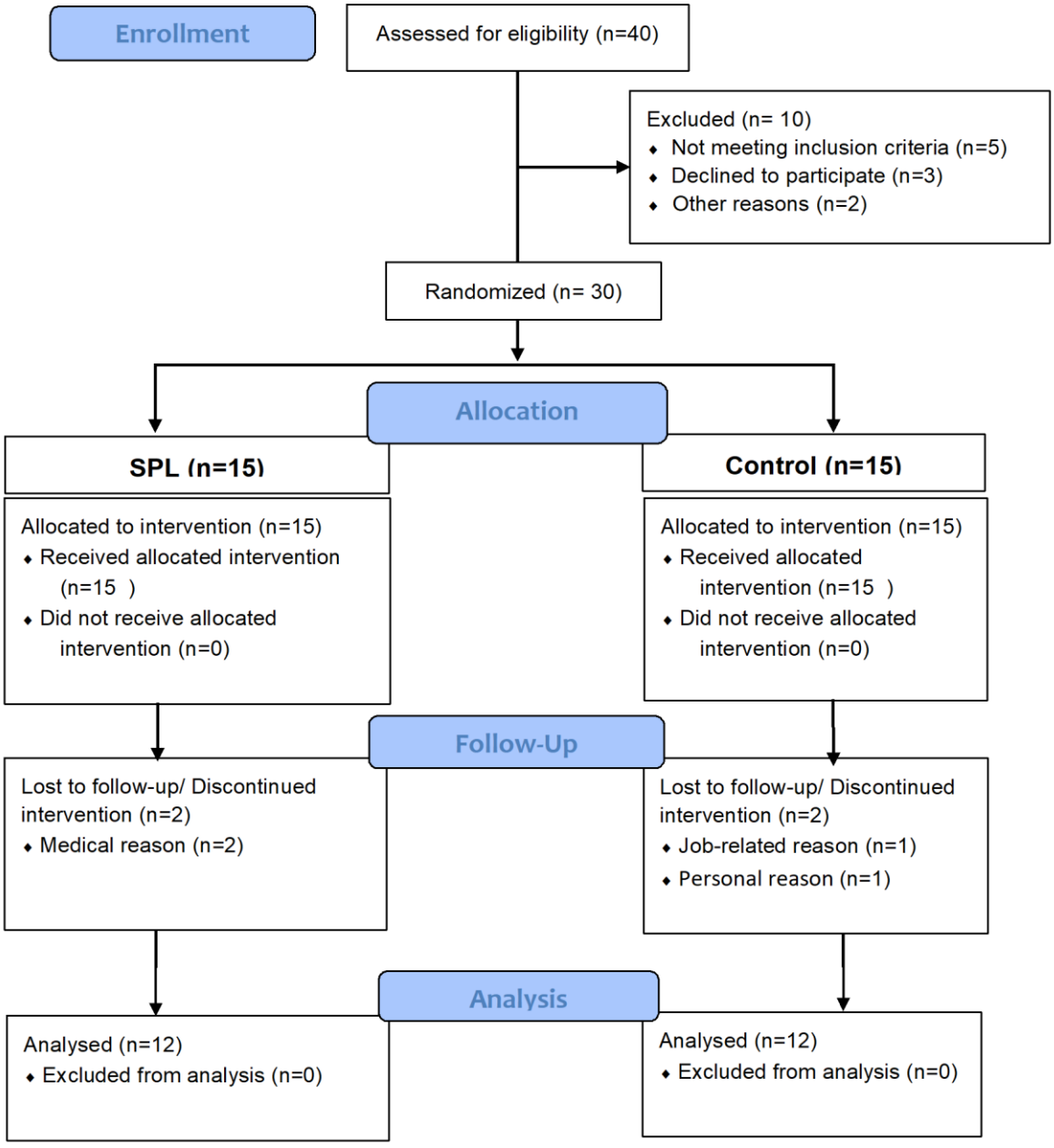

Figure 1. Study flow chart. SPL and control groups.

Table 1: Primer sequences

		Primer
ACE2	**F**	**TCCATTGGTCTTCTGTCACCCG**
	R	**GACCATCCACCTCCACTTCTCT**
NLRP3	**F**	**GGACTCTTGCACCCCGACT**
	R	**GGTCGCCCAGGTCATTGTTG**
FNDC5	**F**	**CGGATTTGCCATCTCCCAGC**
	R	**TTGAAGAGCACAGGCTCGCT**
B2MG (reference)	**F**	**TGAGTCCAAGCTAGGCCCTTT**
	R	**ACCAGCCACCACTTTCTGAT**

SPSS® software). Data were summarized as the mean ± standard deviation. The Kolmogorov-Smirnov test was used to assess the data's normal distribution. T-test was utilized to find differences between the two groups. Furthermore, the Mann-Whitney U was run on non-normally distributed data. Comparing the variables among three groups (group*time) also was done using a two-way ANOVA test. After finding significant differences, pairwise comparisons were utilized to find the differences. P<0.05 were conventionally regarded as statistically significant.

The research reported in this publication was approved by the ethics committee of Islamic Azad University, Tehran, Iran (IR.IAU.TNB.REC.1400.102).

Results

The CONSORT flowchart was used to show the participant flow (Figure 1). Of a total of 40 cases screened in the study, 10 were not eligible based on the inclusion criteria (declined to take part [n=3], not meet inclusion criteria [n=5], and others [n=2]), 30 subjects were allocated to the groups (n=15 in each group). In addition, 2 subjects could not be followed-up in each group due to loss of follow-up/discontinued intervention, and medical reasons. There were no differences in the baseline features of the two groups (P>0.05, Table 3).

Anthropometry and Body Composition: According to table 4, no differences were observed in group×time (pre/post) interaction for changes in BW (P= 0.952), BMI (P= 0.979), BEF (P= 0.457), and WHR (P= 0.238) of spirulina group comparing with the control subjects.

Gene expression of ACE2, NLRP3, and FNDC5: The gene expression of ACE2 (Figure 2a), NLRP3 (Figure 2b), and FNDC5 (Figure 2c) were determined in both groups. Administration of spirulina downregulated ACE2 (P<0.05) and NLRP3 (P<0.05) gene expression compared with the Co-group. In addition, downregulation of ACE2 (P<0.05) and NLRP3 (P<0.05) were observed in pre-investigation values relative to the post-investigation values of the SPL group. Furthermore, FNDC5 gene expression (P<0.05) was increased in the SPL group compared with the control group. The upregulation of FNDC5 was observed in pre-investigation values relative to the post-investigation values of the SPL group (P<0.05).

Correlation between quantitative data: Table 5 and Figure 3 shows the correlation between all quantitative data of the study. There were significant correlations between BW and BMI (r = 0. 893, P<0.0001), BFP (r=0. 449, P<0.0001), and WHR (r=0.691, P<0.0001). In addition, significant positive relationships were seen between BMI and BFP (r=0.619, P<0.0001), and WHR (r=0.744, P<0.0001). Significant correlations were reported between BFP and WHR (r=0.778, P<0.0001). Additionally, significant correlations were found between gene expression of FNDC5 and ACE2 (r= −0.45, P<0.001), and NLRP3 (r= −0.331, P<0.05). There was a significant positive relationship between ACE2 and NLRP3 (r=0.319, P<0.05).

Table 2: Nutritional intake of the study groups.

	Co		SPL	
	Pre (M ± SD)	**Post (M ± SD)**	**Pre (M ± SD)**	**Post (M ± SD)**
Energy (kcal/d)	2358±104	2414±98	2284±76	2237±73
CHO (g/d)	293±14	298±16	281±12	294±14
Fat (g/d)	85.4±8	83.2±10	82.2±9	84.4±8
Protein (g/d)	101±28	102±30	103±34	107±29

M ± SD: Mean± standard deviation, SPL: Spirulina group, Co: Control group

Table 3: Values of baseline characteristics of Co and SPL groups**.**

	Co	**SPL**	**P-value**
	M ± SD	**M ± SD**	
Age	45.00 ± 2.86	44.67 ± 3.34	0.795
Height (m)	1.76 ± 0. 07	1.59 ± 0.09	0.959
BW (Kg)	111.38 ± 24.56	112.83 ± 20.96	0.854
BMI	39.98 ± 7.21	40.66 ± 4.90	0.788

M ± SD: Mean± standard deviation, SPL: Spirulina group, Co: Control group, BW: Body weight, BMI: Body mass index

Discussion

In the present clinical trial, the effects of SPL administration on the body composition measures and the gene expression of FNDC5, ACE2, and NLRP3 were evaluated in the subjects with obesity. The age range of cases was 40 to 50 years and their BMI was more than 30 kg/m^2. According to our results, the administration of SPL (2 g/day) for eight weeks was not effective in normalizing subjects' BW, BMI, BFP, and WHR in obese subjects. SPL is useful in the management of food absorption, insulin resistance, appetite, gut microbiota, oxidative stress, and inflammation, which makes it favorable for obese subjects (22). Several studies showed contradictory results concerning the SPL efficiency on body composition. In a review by Moradi et al. (2019), SPL could reduce the BW, BFP, and WC in obese subjects, but was not effective in reducing BMI and WHR (23). On the other hand, Park and Lee revealed that SPL supplementation could not be effective in reducing the plasma levels of LDL-cholesterol and total cholesterol in obese individuals (24).

The gene expression of NLRP3, ACE2, and FNDC5 was evaluated pre- and post-investigation in obese cases. Obesity strongly increases the risk of respiratory symptoms and infectious diseases. The prevalence of COVID-19 and the severity of disease are higher in obese individuals compared with people

Table 4: Values of body composition and maximal oxygen consumption at pre-and post-intervention.

	Co		**SPL**		**P-value**
	Pre	**Post**	**Pre**	**Post**	
	N=12	**N=12**	**N=12**	**N=12**	
	M ± SD	**M ± SD**	**M ± SD**	**M ± SD**	
BW (kg)	**111.08 ± 24.96**	**106.75 ± 23.16**	**112.83± 20.96**	**107.71 ± 21.93**	**0.952**
BMI (kg/m^2)	**39.98 ± 7.21**	**37.89 ± 7.88**	**40.66 ± 4.90**	**38.47 ± 6.15**	**0.979**
BFP (%)	**48.39 ± 4.86**	**44.77± 2.66**	**46.91 ± 3.47**	**46.91 ± 3.47**	**0.457**
WHR	**1.02 ± 0.09**	**0.97 ± 0.06**	**0.98 ± 0.04**	**0.98 ± 0.09**	**0.238**

M ± SD: Mean± standard deviation, SPL: Spirulina group, Co: Control group, BMI: Body mass index, WHR: Waist-to-hip ratio, VO_{2max}: Maximal oxygen consumption, BFP: Body fat percentage, * P < 0.05 significant differences comparing with the Co group

Table 5: Correlation between quantitative data

		BW (kg)	BMI (kg/m^2)	BFP (%)	WHR	FNDC5	ACE2	NLRP3
BW (kg)	r	1	0.893	0.449	0.691	-0.018	-0.087	-0.104
	P-value	.	<0.0001	<0.0001	<0.0001	0.901	0.557	0.484
BMI (kg/m^2)	r	.	1	0.619	0.744	0.034	0.032	-0.211
	P-value	.	.	<0.0001	<0.0001	0.821	0.828	0.150
BFP (%)	r	.	.	1.000	0.778	0.068	0.130	-0.197
	P-value	.	.	.	<0.0001	0.647	0.380	0.179
WHR	r	.	.	.	1.000	-0.109	0.118	-0.080
	P-value	.	.	.	.	0.459	0.425	0.591
VO_{2max}	r	.	.	.	.	0.058	-0.053	0.209
	P-value	.	.	.	.	0.698	0.720	0.155
FNDC5	r	.	.	.	.	1.000	-0.450	-0.331
	P-value	.	.	.	.	.	0.001	0.022
ACE2	r	.	.	.	.	.	1.000	0.319
	P-value	.	.	.	.	.	.	0.027
NLRP3	r	.	.	.	.	.	.	1.000
	P-value	.	.	.	.	.	.	.

BW: Body weight, BMI: Body mass index BFP: Body fat percentage, WHR: Waist-to-hip ratio

of normal weight (25). Obesity increases the risk of comorbidities to SARS-CoV-2 infection through several mechanisms. In the present study, downregulation of ACE2 was observed in the group receiving SPL and was compared with those of the placebo group. Several reasons may elevate the susceptibility to SARS-CoV-2 infection among obese patients, including weakened immunity and high expression of ACE2 (9). Emilsson et al. (2020) reported higher ACE2 serum levels in patients with obesity and type 2 diabetes (26). In addition, ACE2 has been reposted to be implicated in several pathologic conditions, including diabetes, CVDs, and lung disease (27). SPL, a type of edible microalgae, was shown to reduce the risk of COVID-19 (28). SPL exhibits therapeutic effectiveness against SARS-CoV-2 infection via a wide range of pathways (28, 29). In addition, others have emphasized that 2-14 g of SPL was a safe approach for the treatment of dyslipidemia (30). In line with our findings, Joseph et al. (2020) showed that SARS, MERS, and SARS-CoV-2 entry were inhibited with advanced efficacy when preincubated with spirulina extracts (31). Therefore, regulation of ACE2 via SPL can protect obese patients against SARS-COV-2 entry.

In the present study, SPL supplementation could successfully reduce the gene expression of NLRP3, a crucial component of innate immunity in releasing inflammatory cytokines during SARS-CoV-2 infection (32). In addition, ACE2 was positively correlated with NLRP3 expression. Osborn et al. (2012) found that adipocytes can produce inflammatory mediators in large amounts, including IL-6, IL-12, IL-1β, and tumor necrosis factor-α (TNF-α) (4). The secretion of cytokines is triggered by the NLRP3 inflammasome activation in adipose tissue (33). Additionally, SARS-CoV-2 infections can worsen pre-existing systemic inflammation via the activation of the NLRP3 inflammasome in obese patients (14). Ratajczak et al. (2021) showed that NLRP3 inflammasome was activated following the interactions between the S protein of SARS-CoV-2 and ACE2 (34). The anti-inflammatory effects of SPL are attributed to its ability to regulate several inflammatory components. Per findings reported by Chei et al. (2020), it was stated that SPL extract inhibited the NLRP3 activation by suppressing ERK signaling pathways in RAW264.7 cells (35). In a preclinical study by Mullenix et al. (2021), it was revealed that SPL supplementation reduced circulating inflammatory cytokines (IL-4, IL-3, IL-18, IL-6, and TNF-α), chemokines (CCL20), and NLRP3 gene expression in broilers with a low protein

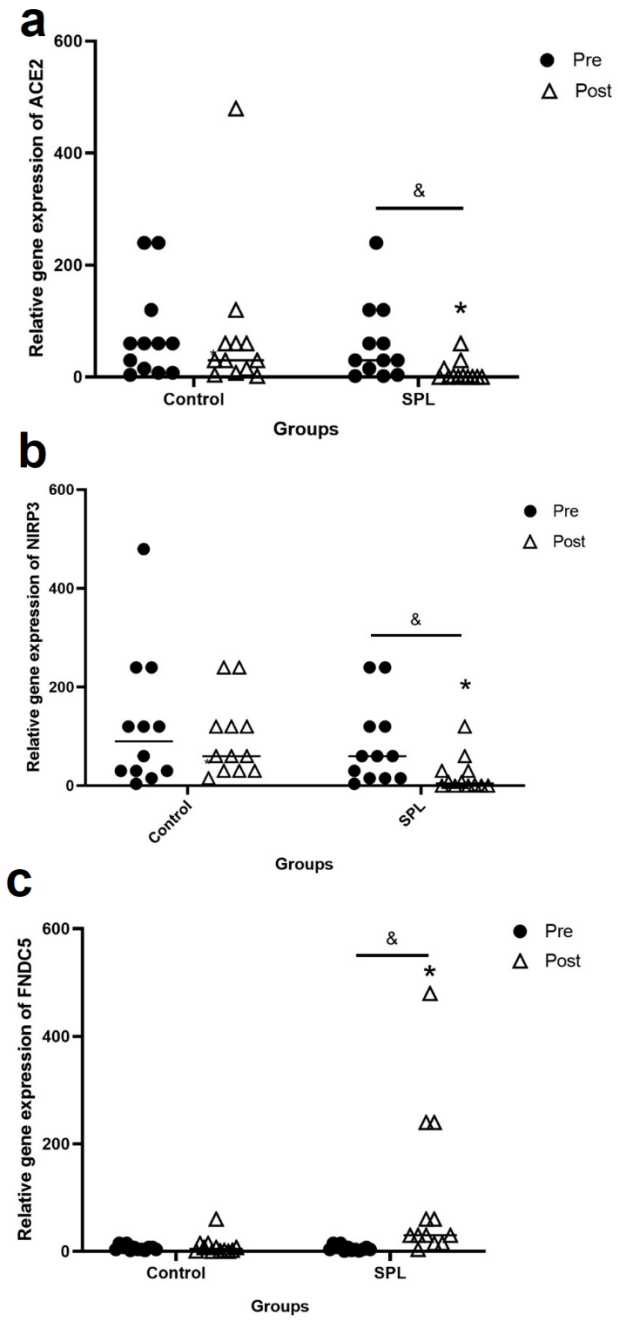

Figure 2. The effects of SPL on the gene expression of a) ACE2, b) NLRP3, and c) FNDC5 in obese subjects. Pre- and post-investigation values (Median). * $P < 0.05$ relative to the Co group, & $P < 0.05$ between pre- and post-investigation values.

diet (36). However, Park and Lee demonstrated that SPL was not effective in the regulation of inflammatory mediators, e.g., IL-2 and Il-6, in obese group (24). Overall, SPL could protect obese patients against SARS-CoV-2 infection via the modulation of ACE2 and NLRP3.

Our results showed that SPL supplementation increased the gene expression of FNDC5. In addition, the expression of FNDC5 was negatively associated with the expression of ACE2 and NLRP3. Therefore,

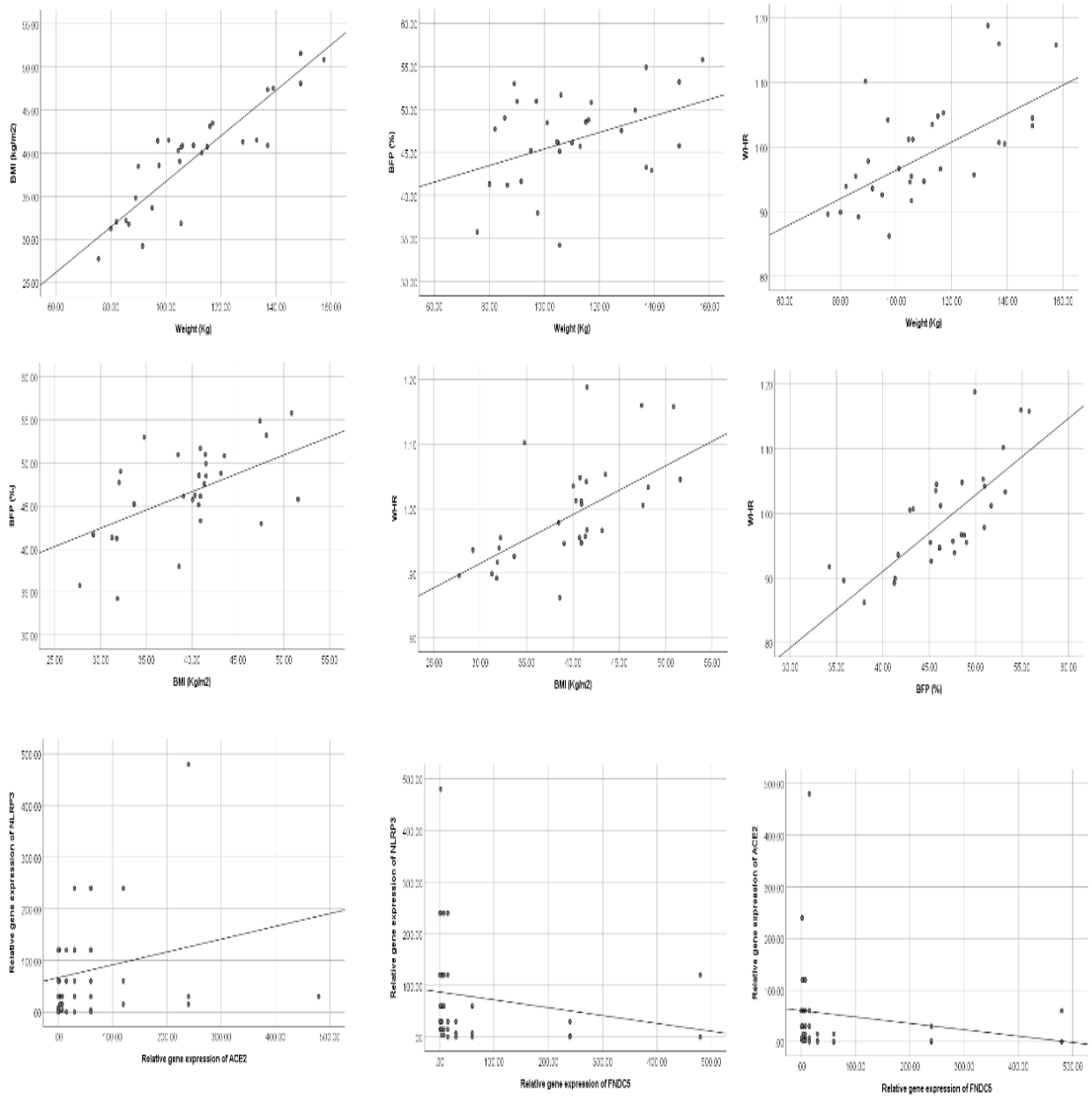

Figure 3. Correlation between quantitative data.

upregulation of FNDC5 can regulate systemic inflammation. Moreno-Navarrete et al. (2013) showed that systemic irisin (encoded by the Fndc5 gene) concentration was negatively related to insulin resistance and obesity (16). FNDC5/irisin produced by adipose tissue is known as an adipokine that can modulate the function of adipocyte action (37). The preclinical investigations have indicated FNDC5/irisin is involved in the induction of the brown fat-like phenotype through the ERK/MAPK signaling pathway (38). To confirm the anti-inflammatory effects, FNDC5 is a regulator of inflammation in the adipose

tissue by inhibiting the polarization of M1 macrophage production of pro-inflammatory cytokines (39). In addition, the antiviral effects of FNDC5 were proven in several studies. de Oliveira et al. (2020) demonstrated that FNDC5 could effectively modulate the SARS-CoV-2 –related gene expression in human adipocytes (20). In addition, Frühbeck et al. (2021) indicated that FNDC5 could reduce ACE2 gene expression in adipocytes. Interestingly, FNDC5 gene silencing increased the gene expression of ACE2 mRNA in human visceral adipocytes. Thus, FNDC5 can inhibit the virus entry into the host cells (17). Taken together, using approaches to increase the levels of FNDC5/irisin can be beneficial for obesity via antiviral and anti-inflammatory effects. There were limited studies to investigate the effects of SPL on FNDC5/irisin levels. The results of a clinical trial by Dehghani et al. (2020) indicated that circuit resistance training with SPL could improve the plasma levels of irisin, but irisin alone was not effective (40).

Conclusion

In summary, SPL supplementation was not beneficial in the normalization of anthropometric measurements in obese patients. It can be due to their age and BMI that can have difficulty responding to the treatment with SPL. Nevertheless, SPL could successfully modulate the gene expression of ACE2, NLRP3, and FNDC5 in patients with obesity. These findings indicate that SPL can protect obese individuals against obesity-induced complications via the regulation of inflammation. In addition, SPL supplementation can prevent COVID-19 by inhibiting the invasion of the virus into the host cells via the downregulation of ACE2. In light of these findings, it would be of particular relevance to further investigate the effects of SPL in overweight and obesity.

Acknowledgment

None.

Conflicts of Interest

The authors declare that they have no conflict of interest.

References

1. Hruby A, Hu FB. The Epidemiology of Obesity: A Big Picture. Pharmacoeconomics. 2015;33(7):673-89.
2. Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. Int J Obes (Lond). 2008;32(9):1431-7.
3. Mafort TT, Rufino R, Costa CH, Lopes AJ. Obesity: systemic and pulmonary complications, biochemical abnormalities, and impairment of lung function. Multidiscip Respir Med. 2016;11:28.
4. Osborn O, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. Nat Med. 2012;18(3):363-74.
5. Vas P, Hopkins D, Feher M, Rubino F, M BW. Diabetes, obesity and COVID-19: A complex interplay. Diabetes Obes Metab. 2020;22(10):1892-6.
6. Kassir R. Risk of COVID-19 for patients with obesity. Obes Rev. 2020;21(6):e13034.
7. Singh A, Roy A, Khanna P. Perioperative consideration of morbid obesity during COVID-19 outbreak. J Cell Mol Anesth. 2020;5(3):185-9.
8. Rajaei S, Dabbagh A. The immunologic basis of COVID-19: a clinical approach. J Cell Mol Anesth. 2020;5(1):37-42.
9. Frasca D, Blomberg BB, Paganelli R. Aging, Obesity, and Inflammatory Age-Related Diseases. Front Immunol. 2017;8:1745.
10. Kuba K, Imai Y, Ohto-Nakanishi T, Penninger JM. Trilogy of ACE2: a peptidase in the renin-angiotensin system, a SARS receptor, and a partner for amino acid transporters. Pharmacol Ther. 2010;128(1):119-28.
11. Engeli S, Böhnke J, Gorzelniak K, Janke J, Schling P, Bader M, et al. Weight loss and the renin-angiotensin-aldosterone system. Hypertension. 2005;45(3):356-62.
12. Esser N, L'Homme L, De Roover A, Kohnen L, Scheen AJ, Moutschen M, et al. Obesity phenotype is related to NLRP3 inflammasome activity and immunological profile of visceral adipose tissue. Diabetologia. 2013;56(11):2487-97.
13. Mokhtari T, Hassani F, Ghaffari N, Ebrahimi B, Yarahmadi A, Hassanzadeh G. COVID-19 and multiorgan failure: A narrative review on potential mechanisms. J Mol Histol. 2020;51(6):613-28.
14. López-Reyes A, Martinez-Armenta C, Espinosa-Velázquez R, Vázquez-Cárdenas P, Cruz-Ramos M, Palacios-Gonzalez B, et al. NLRP3 Inflammasome: The Stormy Link Between Obesity and COVID-19. Frontiers in immunology. 2020;11:570251.
15. Bubak MP, Heesch MWS, Shute RJ, Dinan NE, Laursen TL, DT LAS, et al. Irisin and Fibronectin Type III Domain-Containing 5 Responses to Exercise in Different Environmental Conditions. Int J Exerc Sci. 2017;10(5):666-80.
16. Moreno-Navarrete JM, Ortega F, Serrano M, Guerra E, Pardo G, Tinahones F, et al. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. J Clin Endocrinol Metab. 2013;98(4):E769-78.
17. Frühbeck G, Catalán V, Valentí V, Moncada R, Gómez-

Ambrosi J, Becerril S, et al. FNDC4 and FNDC5 reduce SARS-CoV-2 entry points and spike glycoprotein S1-induced pyroptosis, apoptosis, and necroptosis in human adipocytes. Cell Mol Immunol. 2021;18(10):2457-9.
18. Karkos PD, Leong SC, Karkos CD, Sivaji N, Assimakopoulos DA. Spirulina in clinical practice: evidence-based human applications. Evid Based Complement Alternat Med. 2011;2011:531053.
19. Ngo-Matip ME, Pieme CA, Azabji-Kenfack M, Moukette BM, Korosky E, Stefanini P, et al. Impact of daily supplementation of Spirulina platensis on the immune system of naïve HIV-1 patients in Cameroon: a 12-months single blind, randomized, multicenter trial. Nutr J. 2015;14:70.
20. de Oliveira M, De Sibio MT, Mathias LS, Rodrigues BM, Sakalem ME, Nogueira CR. Irisin modulates genes associated with severe coronavirus disease (COVID-19) outcome in human subcutaneous adipocytes cell culture. Mol Cell Endocrinol. 2020;515:110917.
21. Armannia F, Ghazalian F, Shadnoush M, Keyvani H, Gholami M. Effects of High-Intensity Interval Vs. Moderate-Intensity Continuous Training on Body Composition and Gene Expression of ACE2, NLRP3, and FNDC5 in Obese Adults: A Randomized Controlled Trial. Med J Islam Repub Iran. 2022;36(1):1256-64.
22. DiNicolantonio JJ, Bhat AG, J OK. Effects of spirulina on weight loss and blood lipids: a review. Open Heart. 2020;7(1):e001003.
23. Moradi S, Ziaei R, Foshati S, Mohammadi H, Nachvak SM, Rouhani MH. Effects of Spirulina supplementation on obesity: A systematic review and meta-analysis of randomized clinical trials. Complement Ther Med. 2019;47:102211.
24. Park HJ, Lee HS. The influence of obesity on the effects of spirulina supplementation in the human metabolic response of Korean elderly. Nutr Res Pract. 2016;10(4):418-23.
25. Cai Q, Chen F, Wang T, Luo F, Liu X, Wu Q, et al. Obesity and COVID-19 Severity in a Designated Hospital in Shenzhen, China. Diabetes Care. 2020;43(7):1392-8.
26. Emilsson V, Gudmundsson EF, Aspelund T, Jonsson BG, Gudjonsson A, Launer LJ, et al. Serum levels of ACE2 are higher in patients with obesity and diabetes. Obes Sci Pract. 2021;7(2):239-43.
27. Oudit GY, Imai Y, Kuba K, Scholey JW, Penninger JM. The role of ACE2 in pulmonary diseases--relevance for the nephrologist. Nephrol Dial Transplant. 2009;24(5):1362-5.
28. Siedenburg JR, Cauchi JP. Spirulina (Arthrospira spp) as a Complementary COVID-19 Response Option: Early Evidence of Promise. Curr Res Nutr Food Sci. 2022;10(1):129-44.
29. Tzachor A, Rozen O, Khatib S, Jensen S, Avni D. Photosynthetically Controlled Spirulina, but Not Solar Spirulina, Inhibits TNF-α Secretion: Potential Implications for COVID-19-Related Cytokine Storm Therapy. Mar Biotechnol (NY). 2021;23(1):149-55.
30. Yousefi R, Mottaghi A, Saidpour A. Spirulina platensis effectively ameliorates anthropometric measurements and obesity-related metabolic disorders in obese or overweight healthy individuals: A randomized controlled trial. Complement Ther Med. 2018;40:106-12.
31. Joseph J, Karthika T, Das VRA, Raj VS. The use of Pseudotyped Coronaviruses for the Screening of Entry Inhibitors: Green Tea Extract Inhibits the Entry of SARS-CoV-1, MERSCoV, and SARS-CoV-2 by Blocking Receptor-spike Interaction. Curr Pharm Biotechnol. 2022;23(8):1118-29.
32. Mohamed Khosroshahi L, Rokni M, Mokhtari T, Noorbakhsh F. Immunology, immunopathogenesis and immunotherapeutics of COVID-19; an overview. Int Immunopharmacol. 2021;93:107364.
33. Wani K, AlHarthi H, Alghamdi A, Sabico S, Al-Daghri NM. Role of NLRP3 Inflammasome Activation in Obesity-Mediated Metabolic Disorders. Int J Environ Res Public Health. 2021;18(2).
34. Ratajczak MZ, Bujko K, Ciechanowicz A, Sielatycka K, Cymer M, Marlicz W, et al. SARS-CoV-2 Entry Receptor ACE2 Is Expressed on Very Small CD45(-) Precursors of Hematopoietic and Endothelial Cells and in Response to Virus Spike Protein Activates the Nlrp3 Inflammasome. Stem Cell Rev Rep. 2021;17(1):266-77.
35. Chei S, Oh HJ, Song JH, Seo YJ, Lee K, Kim KJ, et al. Spirulina maxima extract prevents activation of the NLRP3 inflammasome by inhibiting ERK signaling. Sci Rep. 2020;10(1):2075.
36. Mullenix GJ, Greene ES, Emami NK, Tellez-Isaias G, Bottje WG, Erf GF, et al. Spirulina platensis Inclusion Reverses Circulating Pro-inflammatory (Chemo)cytokine Profiles in Broilers Fed Low-Protein Diets. Front Vet Sci. 2021;8:640968.
37. Roca-Rivada A, Castelao C, Senin LL, Landrove MO, Baltar J, Belén Crujeiras A, et al. FNDC5/irisin is not only a myokine but also an adipokine. PLoS One. 2013;8(4):e60563.
38. Rabiee F, Lachinani L, Ghaedi S, Nasr-Esfahani MH, Megraw TL, Ghaedi K. New insights into the cellular activities of Fndc5/Irisin and its signaling pathways. Cell Biosci. 2020;10:51.
39. Xiong XQ, Geng Z, Zhou B, Zhang F, Han Y, Zhou YB, et al. FNDC5 attenuates adipose tissue inflammation and insulin resistance via AMPK-mediated macrophage polarization in obesity. Metabolism. 2018;83:31-41.
40. Malekaneh M, Dehghani K, Mogharnasi M, Saghebjoo M, Sarir H, Nayebifar S. The Combinatory Effect of Spirulina Supplementation and Resistance Exercise on Plasma Contents of Adipolin, Apelin, Ghrelin, and Glucose in Overweight and Obese Men. Mediators Inflamm. 2022;2022:9539286.