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A Randomized Double-Blind, Placebo-Controlled Study to Establish the Effects of Spirulina in Elderly Koreans

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Key Words
Spirulina supplementation · Antioxidant status · Lipid profile · Immune response · Aging

Abstract
Aims: This study was conducted to determine the antioxidant capacity, immunomodulatory and lipid-lowering effects of spirulina in healthy elderly subjects and to document the effectiveness of spirulina as a functional food for the elderly. ***Methods:*** A randomized double-blind, placebo-controlled study was performed. The subjects were 78 individuals aged 60–87 years and were randomly assigned in a blinded fashion to receive either spirulina or placebo. The elderly were instructed to consume the spirulina or placebo at home, 8 g/day, for 16 consecutive weeks. ***Results:*** In male subjects, a significant plasma cholesterol-lowering effect was observed after the spirulina intervention ($p < 0.05$). Spirulina supplementation resulted in a significant rise in plasma interleukin (IL)-2 concentration, and a significant reduction in IL-6 concentration. A significant time-by-treatment intervention for total antioxidant status was observed between spirulina and placebo groups ($p < 0.05$). In female subjects, significant increases in IL-2 level and superoxide dismutase activity were observed ($p < 0.05$) after spirulina supplementation. There were significant reductions in total cholesterol in female subjects. ***Conclusions:*** The results demonstrate that spirulina has favorable effects on lipid profiles, immune variables, and antioxidant capacity in healthy, elderly male and female subjects and is suitable as a functional food.

Introduction

Spirulina is a microscopic and filamentous cyanobacterium (blue-green alga) that has a long history of use as food. Spirulina is a rich source of vitamins, especially vitamin B_{12} and provitamin A (β-carotene), minerals, carotenoids, and phycocyanins [1]. Its safety for human consumption has been established through numerous toxicological studies [2–4].

Recently, spirulina has received attention regarding its association with cholesterol-regulatory properties, modulation of the host immune system, and antioxidant capacity [5–10]. In a spirulina supplementation study involving 15 diabetic patients, Mani et al. [11] found a sig-

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nificant reduction in plasma levels of total lipids, free fatty acids, and triglycerides. A reduction in the LDL/HDL ratio was also observed. In addition, Benedetti et al. [12] demonstrated the antioxidant properties of spirulina in a report showing that an alcohol extract of spirulina inhibited lipid peroxidation more significantly (65% inhibition) than chemical antioxidants such as α-tocopherol (35%), butylated hydroxyanisole (45%), and β-carotene (48%) in vitro. In vivo, C-phycocyanin from spirulina effectively inhibited CCL_4-induced lipid peroxidation in rat liver [13].

Hayashi et al. [14] were the first to publish detailed studies on the immunomodulatory properties of dietary spirulina in mice. In their study, mice fed spirulina showed increased numbers of splenic antibody-producing cells in the primary immune response to sheep red blood cells and an increased percentage of phagocytic cells among peritoneal macrophages. The proliferation of spleen cells induced by either concanavalin A or phytohemagglutinin was also significantly increased in mice fed a spirulina diet and a spirulina extract-enhanced interleukin (IL)-1 production from peritoneal macrophages.

Lipid peroxidation, dysregulated lipid metabolism, and chronic inflammation are major characteristics of age-related changes [15–17] and are considered to be involved in the pathogenesis of all age-associated diseases such as Alzheimer's disease, atherosclerosis, diabetes, and cancer. With advancing age, the balance between reactive oxygen species and the antioxidant system is altered. Overproduction of free radicals causes a cascade of intracellular events, resulting in the liberation into the cytoplasm of nuclear transcription factor κB (NF-κB) from its inhibitory protein IκB. NF-κB controls the production of acute-phase mediators such as tumor necrosis factor-α (TNF-α), IL-2, and IL-2 receptors, which in turn activate NF-κB, amplifying the inflammatory cascade. Therefore, cross talk between lipid peroxidation and inflammation needs to be considered in the aging process.

Recently, functional foods have been actively sought to control and prevent aging and age-associated diseases. Based on epidemiological and clinical trials [18–23], spirulina appeared to suit its purpose. However, few studies have examined the effect of spirulina in elderly subjects using double-blind, placebo-controlled trials. This study was conducted to examine the immunomodulatory, antioxidant capacity and lipid-lowering effects of spirulina in healthy elderly subjects and to document the effectiveness of spirulina as a functional food for the elderly.

Subjects and Methods

Subjects and Experimental Design

The subjects for this double-blind, placebo-controlled intervention study were recruited between December 2005 and June 2006 through advertisements in a local newspaper. Volunteers aged >60 years were first interviewed by telephone for screening (n = 97). The exclusion criteria were current use of vitamin supplements, current use of drugs for inflammatory disease (e.g., Crohn's disease, rheumatoid arthritis), dyslipidemia, hypertension, and concurrent or recent participation in other intervention studies. 78 subjects (43 male, 35 female) were enrolled. The study protocol was approved by the Institutional Review Board of Ewha Womans University Medical Center. All subjects gave written informed consent before beginning the study and were free to withdraw from the study at any time without obligation. The participants received pills containing 0.2 g of freeze-dried spirulina or the equivalent amount of pure starch. They were instructed to consume 40 pills daily to supply 8 g/day of spirulina or placebo at home for 16 consecutive weeks. Subjects were required to abstain from taking any other supplements or any other medication during the study period without consulting the investigators. Both spirulina and placebo (100% starch, pills) were provided by the Earth Spirulina Group (ES Co., Korea).

At each subject's first visit, baseline blood samples were drawn after a minimum of 12 h of fasting. Blood samples were taken again at the end of the study period of 4 months. Anthropometric parameters and dietary intake were also measured at each visit. The spirulina and placebo were sent to the subjects every 2 weeks and compliance was confirmed by telephone twice a week.

Subjects' Baseline Characteristics

The subjects were individually interviewed to obtain food consumption data, general characteristics, and lifestyle behavior. Food consumption was assessed using the 24-hour recall method. Food models were used to estimate portion sizes. Food intake data was analyzed using CAN-Pro 3.0 [24], computerized nutrient intake assessment software developed by the Korean Nutrition Society.

Standing height was measured using an anthropometer (Seca, Inc., Germany). Body weight and body composition [body fat (kg), body fat (%), and lean body mass] were measured using INBODY 2.0 (Biospace Co., Korea), with subjects wearing light clothing without shoes or socks. Waist and hip circumferences were measured using a tape measure (Anthropometric Tape, Sammons' Preston Model 5193, USA); waist circumference was measured midway between the lowest rib margin and the iliac crest at the end of gentle expiration. Body mass index (BMI kg/m^2) and waist-to-hip ratio (WHR) were calculated. Triceps skinfold thickness (TSF) was measured using a Lange skinfold caliper (Cambridge Scientific, Inc., USA). Sitting systolic and diastolic blood pressures were measured twice using an automatic blood pressure calculator (Omron, HEM-705, Japan) after a 10-min rest in the sitting position; the average of the two measurements was used.

Determination of Plasma Lipid Profiles

Total cholesterol and triglyceride levels were assessed using an autoanalyzer (Ektachem DTSC module, Johnson & Johnson, USA). HDL cholesterol levels were determined by autoanalyzer

Table 1. Baseline characteristics (mean ± SE) of the subjects enrolled in the intervention study

	Male (n = 43)		Female (n = 35)	
	spirulina (n = 24)	placebo (n = 19)	spirulina (n = 17)	placebo (n = 18)
Age, years	66.1 ± 1.2	66.6 ± 1.1	65.6 ± 1.4	65.4 ± 1.6
Cigarette smoking, yes %	12.5	21.1	5.9	0
Alcohol drinking, yes %	66.7	78.9	29.4	5.7
Anthropometric values				
Weight, kg	62.7 ± 3.2	63.6 ± 2.7	63.5 ± 2.4	63.2 ± 2.6
BMI, kg/m^2	24.8 ± 0.7	24.6 ± 0.5	23.9 ± 0.5	24.1 ± 0.7
WHR	0.94 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	0.93 ± 0.01
TSF, mm	22.3 ± 1.8	17.8 ± 1.5	30.9 ± 1.6	29.9 ± 1.6
Body fat, %	25.4 ± 1.1	25.5 ± 0.9	32.6 ± 1.0	33.0 ± 1.0
Diet intakes				
Energy, kcal/day	1,575.9 ± 86.1	1,501.5 ± 116.4	1,583.3 ± 110.9	1,579.1 ± 115.5
Protein, g/day	64.0 ± 4.2	60.5 ± 5.8	67.1 ± 5.8	62.8 ± 5.3
Fat, g/day	32.5 ± 2.6	31.6 ± 4.0	40.2 ± 5.2	34.2 ± 4.3
Carbohydrate, g/day	256.3 ± 15.2	243.7 ± 18.1	223.9 ± 14.5	243.5 ± 15.9
Plasma values				
Fasting blood sugar, mg/dl	106.04 ± 2.8	106.1 ± 5.3	103.8 ± 3.6	95.6 ± 2.0
Total cholesterol, mg/dl	181.5 ± 7.1	179.1 ± 7.9	200.5 ± 9.4	213.7 ± 8.0
LDL cholesterol, mg/dl	109.3 ± 7.9	112.9 ± 7.6	126.7 ± 8.3	139.8 ± 10.9
HDL cholesterol, mg/dl	50.5 ± 4.1	46.1 ± 3.8	46.8 ± 1.8	52.6 ± 4.0
Triglyceride, mg/dl	108.2 ± 13.8	100.6 ± 10.1	135.5 ± 23.9	106.7 ± 14.7
Blood pressure				
SBP, mm Hg	139.0 ± 3.4	143.7 ± 3.7	130.8 ± 2.9	128.7 ± 3.5
DBP, mm Hg	85.3 ± 2.0	89.5 ± 1.8	80.4 ± 2.5	80.3 ± 1.4

BMI = Body mass index; WHR = waist-to-hip ratio; TSF = triceps skin fold thickness; SBP = systolic blood pressure; DBP = diastolic blood pressure.

after treating ultracentrifugation infranatants with phosphotungstic acid-Mg. LDL cholesterol and atherogenic index (AI) were calculated as using the Friedewald [25] and Lauer [26] equations, respectively.

Determination of Plasma Immunological Parameters

Plasma levels of IL-2, IL-6, TNF-α, and monocyte chemoattractant protein-1 (MCP-1) were determined by enzyme-linked immunosorbent assay (ELISA) techniques (Quantikine, R&D Systems, Inc., USA) read using an ELISA reader (Spectra Max 340, USA). C3 concentration was determined using radial immunodiffusion plates (Norpartigen, Behring Co., Germany).

Plasma Levels of Antioxidant Parameters

Plasma thiobarbituric acid-reactive substance (TBARS) concentration was determined by the Yagi method [27] using a luminescence spectrometer (PerkinElmer, LS50, USA) with excitation at 515 nm and emission at 553 nm. A standard curve was constituted from serial dilutions (0–1.0 nM) of a 1,1,3,3-tetra-methoxypropane[malonaldehyde bis(dimethyl acetal)] standard solution. The total antioxidant status (TAS) of plasma samples was assessed using a commercial TAS kit (Randox Laboratories Ltd, UK). Plasma superoxide dismutase (SOD) activity was measured by the Maklund and Sheri method [28, 29], and the level of glutathione peroxidase (GPx) was measured using a GPx-EIA assay kit (OXIS International, Inc., USA).

Statistical Analysis

Statistics analyses were conducted using the SAS 9.1 program. Data are presented as means ± SE. Paired t tests were used to analyze mean differences between baseline and 4 months for all measured parameters. Data within each group were analyzed by repeated measures analysis of variance to establish significant differences in treatment. All comparisons were done at the 5% level of significance.

Results

No statistically significant differences in baseline characteristics between spirulina and placebo groups for either gender were found for age, cigarette smoking and alcohol consumption, anthropometry, blood pressure, diet intake, or plasma lipids concentration (table 1). During the 4 months of the intervention period, no signifi-

Table 2. Plasma lipid profiles (mean ± SE) of the subjects during the intervention period

	Male					Female				
	spirulina (n = 24)		placebo (n = 19)			spirulina (n = 17)		placebo (n = 18)		
	baseline	4 months	baseline	4 months	p	baseline	4 months	baseline	4 months	p
Total cholesterol, mg/dl	181.5 ± 7.1	175.9 ± 8.4	179.1 ± 7.9	184.5 ± 9.7	0.0292	200.5 ± 9.4	184.8 ± 6.4*	213.7 ± 8.0	210.4 ± 9.3	0.0387
LDL cholesterol, mg/dl	109.7 ± 8.6	110.2 ± 9.2	112.9 ± 7.6	115.9 ± 9.6	0.2034	126.7 ± 8.3	112.1 ± 6.7*	139.8 ± 10.9	135.3 ± 10.9	0.0541
HDL cholesterol, mg/dl	50.5 ± 4.1	46.8 ± 2.7	46.1 ± 3.8	47.7 ± 3.9	0.4375	46.8 ± 1.8	47.1 ± 2.9	52.6 ± 4.0	53.6 ± 3.7	0.6944
LDL/HDL ratio	2.43 ± 0.21	2.68 ± 0.19	2.72 ± 0.26	2.8 ± 0.29	0.1478	2.78 ± 0.22	2.64 ± 0.28	2.97 ± 0.3	2.29 ± 0.27	0.3855
Triglyceride, mg/dl	106.5 ± 13.8	104.5 ± 13.4	100.6 ± 10.1	104.2 ± 14.9	0.2222	135.5 ± 23.9	128.2 ± 23.2	106.7 ± 14.7	107.2 ± 15.8	0.9249
AI	2.93 ± 0.24	3.28 ± 0.20	3.21 ± 0.29	3.25 ± 0.31	0.1891	3.39 ± 0.27	3.05 ± 0.30	3.4 ± 0.31	2.8 ± 0.35	0.5263

AI = Atherogenic index. p = Pr > F by repeated measures ANOVA between spirulina and placebo groups of the same gender.
* Significantly different according to paired t test values between baseline and 4 months in the same group by gender ($p < 0.05$).

Table 3. Plasma immune variables (mean ± SE) of the subjects during the intervention period

	Male					Female				
	spirulina (n = 24)		placebo (n = 19)			spirulina (n = 17)		placebo (n = 18)		
	baseline	4 months	baseline	4 months	p	baseline	4 months	baseline	4 months	p
IL-2, pg/ml	9.43 ± 0.21	13.6 ± 0.32*	13.2 ± 0.53	13.0 ± 0.25	<0.0001	9.39 ± 0.28	13.8 ± 0.32*	10.9 ± 0.63	13.3 ± 0.28*	0.0472
IL-6, pg/ml	2.64 ± 1.10	1.94 ± 0.91	1.07 ± 0.25	1.99 ± 0.65	0.0480	1.02 ± 0.17	1.80 ± 0.65*	1.52 ± 0.46	1.65 ± 0.65	0.6287
TNF-α, pg/ml	3.39 ± 1.03	1.03 ± 0.05	1.85 ± 0.78	1.03 ± 0.05	0.2497	1.87 ± 0.60	1.20 ± 0.20	2.82 ± 0.96	1.03 ± 0.06	0.4942
MCP-1, pg/ml	77.6 ± 4.80	77.6 ± 4.57	91.1 ± 8.35	84.3 ± 5.05	0.4149	78.2 ± 5.41	69.5 ± 3.92	70.2 ± 7.36	69.7 ± 4.46	0.6066
C3, g/l	0.67 ± 0.03	0.67 ± 0.02	0.66 ± 0.02	0.73 ± 0.02*	0.0604	0.78 ± 0.02	0.77 ± 0.02	0.67 ± 0.03	0.74 ± 0.03	0.0878

p = Pr > F by repeated measures ANOVA between spirulina and placebo groups of the same gender.
* Significantly different by paired t test between initial and 4-month values in the same group by gender ($p < 0.05$).

cant changes in baseline characteristics occurred in any group (data not shown).

Lipid Profiles during the Intervention Period

In the male subjects, all the measured plasma lipid concentrations were within the normal ranges, as shown in table 2. After 4 months of intervention, no significant changes were observed in the plasma concentrations of LDL cholesterol, HDL cholesterol, or triglycerides in either group. However, spirulina supplementation showed a significant lowering effect on plasma cholesterol by repeated test for treatment (time a treatment interaction, $p < 0.05$). In the female subjects, mean plasma levels of total cholesterol and LDL cholesterol were above the normal range and HDL cholesterol levels were in the normal range. There were significant reductions in total cholesterol (from 200.5 to 184.8 mg/dl) and a 13% reduction in LDL cholesterol (from 126.7 to 112.1 mg/dl) after 4 months of supplementation with spirulina, while no changes occurred in the placebo group. Levels of HDL cholesterol and triglycerides did not change after the intervention in both groups.

Immune Variables during the Intervention Period

In the male subjects, spirulina supplementation resulted in a significant rise (time · treatment interaction, $p < 0.0001$) in IL-2 concentration, and a significant reduction (time · treatment interaction, $p < 0.05$) in IL-6 concentration (table 3). In the female subjects, the levels of IL-2 were significantly increased in both placebo and spirulina-supplemented groups; however, a more favorable effect on IL-2 levels was observed after spirulina supplementation (time · treatment interaction, $p < 0.05$). For all the other measured immune variables, no effects of supplementation with either placebo or spirulina were observed.

Table 4. Plasma antioxidant status (mean ± SE) of the subjects during the intervention period

	Male					Female				
	spirulina (n = 24)		placebo (n = 19)			spirulina (n = 17)		placebo (n = 18)		
	baseline	4 months	baseline	4 months	p	baseline	4 months	baseline	4 months	p
TBARS, nmol/ml	7.8 ± 0.5	5.6 ± 0.5**	5.9 ± 0.2	5.4 ± 0.2	0.0966	6.5 ± 0.4	5.9 ± 0.6*	7.4 ± 0.7	6.5 ± 0.4	0.8996
TAS, nmol/l	1.6 ± 0.1	2.2 ± 0.2**	1.9 ± 0.1	2.0 ± 0.2	0.0316	1.7 ± 0.1	2.1 ± 0.2	1.7 ± 0.1	2.1 ± 0.2	0.4195
SOD, U/mg	1.8 ± 0.3	2.5 ± 0.2	1.9 ± 0.3	2.4 ± 0.3	0.6845	1.6 ± 0.2	2.7 ± 0.3**	1.9 ± 0.3	2.5 ± 0.2	0.0347
GPx, ng/ml	97.3 ± 14.4	107.9 ± 5.4	106.6 ± 15.1	123.9 ± 13.3	0.1115	129.2 ± 10.5	114.9 ± 7.2	96.6 ± 12.3	99.3 ± 4.5	0.8676

Significantly different by paired t-test between initial and 16-week values in same group by sex (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$).
p = Pr > F by repeated measures ANOVA between the spirulina and placebo groups of the same gender.
TBARS = Thiobarbituric acid response substance; TAS = total antioxidant status; SOD = superoxide dismutase; GPx = glutathione peroxidase.

Antioxidant Status during the Intervention Period

In the male subjects, the plasma level of TBARS decreased by 29% (from 7.8 to 5.6 nmol/ml) during spirulina intervention. A significant time-by-treatment intervention for TAS was observed between two groups ($p < 0.05$). In the female subjects, a significant increase in SOD activity was found after spirulina supplementation (time · treatment interaction, $p < 0.05$) (table 4).

Discussion

It has been reported that spirulina has favorable effects on aging, cancer, dyslipidemia, hypertension, and diabetes, just to mention a few among many claims. These claims come from its lipid-lowering effects, immune-enhancing effects, and antioxidant capacity. We have reported the lipid-lowering effect of spirulina in elderly subjects [30]; however, the study suffered from a small sample size and the lack of a control group and did not use a blind protocol. The present study was designed by double-blind, placebo-controlled trial with larger study samples.

The results from this study showed that supplementation with spirulina lowered plasma total cholesterol levels in both male and female subjects and LDL cholesterol in female subjects. It is consistent with our previous study [30], in which the levels of total cholesterol and LDL cholesterol were considerably decreased in 12 elderly after spirulina supplementation for 24 weeks. Many other animal and human studies have also repeatedly reported the lipid-lowering effects of spirulina. In a study of 30 male volunteers with mild hyperlipidemia and mild hypertension [31], spirulina supplementation at 4.2 g/day for 4 weeks resulted in a significant reduction in LDL cholesterol and AI; however, LDL cholesterol increased back to its baseline value 4 weeks after discontinuing spirulina administration. De Rivera et al. [32] measured liver levels of triglycerides and phospholipids in rats fed a 60% fructose diet supplemented with 5% spirulina. Rats fed the spirulina diet had lower levels of liver triglycerides and phospholipids compared to rats fed 60% fructose diet without spirulina supplementation. In addition, rats fed 20% water-soluble spirulina fraction showed raised HDL to LDL ratios and lower fasting serum glucose levels [33].

Spirulina has several antioxidant components, such as vitamin B_{12}, β-carotene, tocopherol, carotenoids, and phycocyanin. We examined the plasma levels of TBARS, TAS, SOD, and GPx as antioxidant capacity biomarkers. We observed that antioxidant capacity improved, as shown by an increasing level of TAS and decreasing TBARS after spirulina supplementation. An in vitro study by Miranda et al. [34] revealed that peroxidation of rat brain homogenates was inhibited by almost 95% following treatment with 0.5 mg of methanolic spirulina extract. In vivo antioxidant capacity was evaluated in the plasma of animals receiving a daily dose of 5 mg spirulina extract for 2 and 7 weeks. Upon treatment, the antioxidant capacity of the plasma was 97 and 71% in the experimental group and 74 and 54% in the control group after 2 and 7 weeks, respectively [34].

Cytokines are proteins produced by lymphocytes that control the behavior of immune cells. As cytokines are typically multifunctional, analysis of several cytokines allows a more fundamental understanding of the immune system. A group of researchers [35] using human peripheral blood mononuclear cells demonstrated that

spirulina stimulates the secretion of interleukin-1β, IL-4, and interferon-γ to nearly 2.0, 3.3, and 13.6 times the basal levels, respectively. In our study, plasma IL-2 levels were increased after spirulina supplementation in both gender groups. IL-2 is an anti-inflammatory cytokine and is an essential regulator of chronic inflammatory responses [36]. As plasma IL-2 levels are known to decrease with aging, raising plasma IL-2 levels is important for proper immune regulation in elderly people [37, 38]. Even though the reductions in the levels of proinflammatory cytokines such as TNF-α, MCP-1, and C3 were not significant after supplementation with spirulina, the sum of downregulating effect of proinflammatory cytokines could be meaningful. Moreover, the ratio of anti- and proinflammatory factors could be changed, resulting in a reduced inflammatory response.

In summary, our double-blind, placebo-controlled study confirmed previous reports that spirulina has effects on lipid profiles, immune variables, and antioxidant capacity. These results suggest that spirulina is a promising agent as a functional food for elderly people. Further studies are needed to ascertain the mechanism of spirulina's actions on lipid profiles, immune variables, and antioxidant capacity.

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