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The influence of obesity on the effects of spirulina supplementation in the human metabolic response of Korean elderly

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BACKGROUND/OBJECTIVES: Spirulina, a blue-green alga, is widely produced and commercialized as a dietary supplement with bio- and immune-modulatory functions. We have previously shown that spirulina had favorable effects on lipid profiles, immune functions, and antioxidant capacity in healthy Korean elderly. Despite favorable effect of spirulina supplementation, some sub-populations have shown a poor response to supplementation. Obesity is a factor related to poor-response. Therefore, the purpose of this study was to determine the immuno-modulation, antioxidant capacity, and lipid-lowering effect of spirulina in obese and non-obese Korean elderly.
SUBJECTS/METHODS: The subjects were 78 elderly aged 60-87 years. In a randomized double blind, placebo-controlled study, subjects were fed either placebo or spirulina daily, at 8 g for 12 weeks. Subjects were divided into the non-obese group and the obese group based on body mass index (BMI) criteria for Asians suggested by the International Obesity Task Force: BMI < 25 kg/m^2 (non-obese) and BMI ≥ 25 kg/m^2 (obese).
RESULTS: In the non-obese group, spirulina supplementation showed a significant lowering effect on plasma concentration of total cholesterol and LDL-cholesterol, a significant increase in interleukin (IL)-2 concentration ($P < 0.01$) and a significant increment ($P < 0.05$) in IL-2/IL-6 ratio, and a significant increase in total antioxidant status level and a significant decrease in thiobarbituric acid reactive substances level. However, these effects were not observed in the obese group.
CONCLUSION: These results demonstrated that blood lipid lowering and immune and antioxidant improving response for spirulina supplement was affected by obesity in Korean elderly.

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INTRODUCTION

Spirulina representing a blue-green alga is a filamentous cyanobacterium consumed by humans for hundreds of years as food or as dietary supplements [1]. Spirulina is regarded as one of the most prophylactic and healing nutritional ingredients in the 21st century [2] due to its nutrient profile, lack of significant side-effects [3], and therapeutic effects [4,5]. Spirulina contains high quality protein, minerals (K, Ca, Mg, Fe, Zn, Na), vitamins, particularly vitamin B_{12} and provitamin (β-carotene), polyunsaturated fatty acids and other bioactive molecules including phenolic acids, tocopherols, and γ-linolenic acid [3,6]. Spirulina is widely produced and commercialized as a dietary supplement for treatment of malnutrition and modulating immune functions, as well as ameliorating a variety of diseases [1] including obesity, hypercholesterolemia, arterial hypertension [7], insulin-resistance [8], diabetes mellitus [6,9] and non-alcoholic fatty liver disease [10]. In addition, some studies have shown that spirulina has beneficial effects on the reduction of drug toxicity [11] and immunostimulant effects [12,13]. We have already reported on the effect of spirulina for lipid lowering, immune response, and antioxidant capacity in Korean elderly [14]. However, obese individuals have systemic markers for chronic low-grade inflammation and have a greater risk for chronic disease such as metabolic syndrome, diabetes, and cardiovascular disease. Many studies have reported that oxidative stress is involved in obesity, in addition to many other human diseases and aging [15,16]. A recent study reported that the immune system is adversely affected by obesity, and these "immune consequences" raise concern for the lack of vaccine-induced immunity in obese patients [17]. It means poor immune response in obese subjects. Bozaoqlu *et al*. [18] reported that plasma soluble interleukin-1 receptor accessory protein level is reduced in obesity, which is involved in the signaling pathway in chronic allergies, atherosclerosis, and rheumatoid arthritis. A recent study in obese mice demonstrated that lung pathology and decreased survival following influenza infection is, in part, due to impaired M2 macrophage function [19] In addition,

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previous studies in younger participants have suggested that lysine may have a beneficial effect on glucose metabolism. However, acute lysine supplementation in the older population or overweight subjects, with poor immune response, does not facilitate beneficial changes in glucose Ra or glucose Rd [20]

Therefore, we were interested in the point that food supplements might be influenced by the effect of body mass index (BMI). Few studies have examined the effect of food supplementation by BMI. For this purpose, we analyzed the immunomodulation, antioxidant capacity, and lipid-lowering effect of spirulina supplementation by BMI in Korean elderly.

SUBJECTS AND METHODS

Subjects and experimental design

The subjects for intervention study were recruited through an advertisement in local newspapers (2005.12 ~ 2006.6). The volunteers aged over 60 years were first interviewed by telephone for screening (n = 97). The exclusion criteria were current user of vitamin supplements, current drug-user for inflammatory disease (e.g., Crohn disease, rheumatoid arthritis), dyslipidemia, or hypertension, or concurrent or recent participant in another intervention study. Finally, 78 subjects (male 43, female 35) were enrolled. The protocol was approved by the Institutional Review Board of Ewha Womans University Medical Center (ECT 109-02-01). All subjects gave written informed consent before beginning the study and were free to withdraw from the study at any time without obligation. In this double-blind, placebo-controlled protocol, the subjects were randomly assigned in a blinded fashion to receive either spirulina or placebo for 4 months. The subjects were instructed to consume spirulina or placebo at home, 8 g per day, 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) were provided by Earth Spirulina group (ES co. Korea). Subjects were divided into the non-obese group and the obese group based on BMI criteria for Asians suggested by the International Obesity Task Force: BMI < 25 kg/m^2 (non-obese) and BMI ≥ 25 kg/m^2 (obese).

At the first visit for intervention study, blood was drawn after a minimum of 12 h of fasting, defined as baseline. 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. Spirulina and placebo were supplied every 2 weeks and compliance was confirmed by telephone twice a week.

Baseline subjects characteristics

The elderly subjects were interviewed individually to obtain data on food consumption, general characteristics, and life-style behavior. Food consumption was assessed using 24-hour recall method. Food intake data was analyzed using CAN-pro 3.0 (Korean Nutrition Society, Korea) [21], computerized nutrient intake assessment software developed by the Korean Nutrition Society.

The standing height was measured using an anthropometer (Seca 213, Seca Inc. Birmingham, UK). Body weight and body composition [body fat (kg), body fat (%), and lean body mass] were measured using INBODY 2.0 (Biospace co, Seoul, Korea), with subjects wearing light clothing without shoes or socks. Waist and hip circumferences were measured by a tape-line (Anthropometric tape model 5193, Smmons' Preston, Warrenville, IL, USA) and waist circumference was measured midway between the lowest rib margin and the iliac crest at the end of gentle expiration. BMI (kg/m^2) and waist-to-hip ratio (WHR) were calculated. Triceps skinfolds thickness (TSF) was measured using a Lange skinfolds caliper (Cambridge Scientific Inc., Watertown, MA, USA). The sitting systolic and diastolic blood pressures were measured twice using an automatic blood pressure calculator (HEM-705, Imron, Kyoto, Japan), after a 10-minute rest in the sitting position and the average of the 2 measurements was used.

Determination of plasma lipid profiles

Total cholesterol and triglyceride levels were assessed using an autoanalyzer (Ekachem DTSC module, Johnson&Johnson, New Brunswick, NJ, USA). HDL-cholesterol level was determined using an autoanalyzer after treatment with UC infranatant with phosphotungstic acid-Mg. LDL-cholesterol and atherogenic index (AI) were calculated as described by the Friedewald [22] and Lauer [23] equation, respectively.

Determination of plasma immunological parameters

Plasma levels of interleukin(IL)-2, IL-6, and tumor necrosis factor-α (TNF-α) were determined by enzyme linked immunosorbent assay (ELISA) technique (Quantikine Elisa kit, R&D systems Inc., Minneapolis, MN, USA) reading with an ELISA reader (Spectra Max 340, Molecular Devices, Sunnyvale, CA, USA).

Plasma level of antioxidant parameters

Plasma thiobarbituric acid reactive substance (TBARS) concentration was determined by Yagi method [24] using a luminescence spectrometer (LS 50, Perkin elmer, Waltham, MA, USA) at excitation 515 nm, emission 553 nm. A standard curve was made from serial dilutions (0-1.0 nM) of a 1,1,3,3-tetra-methoxypropane [Malonaldehyde bis (dimethyl-acetyl)] standard solution. Total antioxidant status (TAS) of plasma sample was assessed using a commercial TAS kit (Randox Laboratories Ltd, London, UK).

Statistical analysis

Statistics analyses were performed using SAS 9.4 program (SAS Institute, NC, USA). Data are presented as mean ± SE. Paired t-test was used for analysis of mean differences for all measured parameters between baseline and 4 months. Data within each group were analyzed using repeated measures analysis of variance and Scheffe's post hoc tests to determine significant difference in treatment. Comparisons were done at the 5% level of significance.

RESULTS

Baseline characteristics of the subjects

As shown in Table 1, there were no significant differences in age, anthropometry data, and blood pressure in baseline characteristics between spirulina and placebo groups for either BMI group (Table 1). In the non-obese group, fasting blood

Table 1. Baseline characteristics of the subjects for the intervention study

	Non-obese (BMI < 25 kg/m^2) (n = 45)		Obese (BMI ≥ 25 kg/m^2) (n = 33)	
	Spirulina (n = 25)	Placebo (n = 20)	Spirulina (n = 16)	Placebo (n = 17)
Age (yrs)	66.2 ± 1.3[1)]	66.6 ± 1.5	65.3 ± 1.0	65.3 ± 1.0
Anthropometric values				
Weight (kg)	57.9 ± 1.2	57.1 ± 1.5	74.4 ± 3.0	67.2 ± 1.8
BMI (kg/m^2)[2)]	22.6 ± 0.3	22.5 ± 0.3	27.7 ± 0.6	26.3 ± 0.4
Body fat (%)	27.4 ± 1.2	27.6 ± 1.1	30.1 ± 1.2	30.9 ± 1.3
WHR[3)]	0.85 ± 0.01	0.84 ± 0.01	0.91 ± 0.01	0.89 ± 0.01
TSF (mm)[4)]	25.6 ± 1.7	22.42 ± 1.9	26.3 ± 2.5	25.1 ± 2.2
Diet intakes				
Energy (kcal/day)	1,514.3 ± 84.5	1,437.1 ± 84.0	1,487.6 ± 56.8	1,776.4 ± 158.9*
Protein (g/day)	62.6 ± 4.1	58.3 ± 4.8	61.1 ± 5.6	70.1 ± 6.2
Fat (g/day)	33.9 ± 3.3	29.6 ± 2.7	32.5 ± 3.4	39.9 ± 5.0
Carbohydrate (g/day)	238.8 ± 13.1	238.3 ± 16.0	240.9 ± 12.7	264.5 ± 22.6*
Fiber (g/day)	6.7 ± 0.4	6.9 ± 0.6	7.6 ± 0.5	7.5 ± 0.7
Plasma values				
Fasting blood sugar (mg/dl)	103.6 ± 3.0	95.6 ± 1.9*	107.5 ± 3.0	107.2 ± 5.8
Total-cholesterol (mg/dl)	191.1 ± 6.9	196.0 ± 8.3	186.5 ± 10.3	196.8 ± 9.7
LDL-cholesterol (mg/dl)	120.5 ± 6.6	123.7 ± 11.0	110.1 ± 10.7	129.5 ± 7.7
HDL-cholesterol (mg/dl)	51.5 ± 3.1	53.7 ± 4.2	45.0 ± 4.1	43.8 ± 2.9
Triglyceride (mg/dl)	95.3 ± 13.6	92.4 ± 9.2*	157.3 ± 22.3	117.5 ± 15.6
Blood pressure				
SBP[5)] (mmHg)	130.4 ± 14.2	138.9 ± 3.7	143.6 ± 3.3	138.1 ± 4.3
DBP[6)] (mmHg)	80.3 ± 9.3	82.6 ± 1.8	87.8 ± 2.4	87.7 ± 1.9

[1)] Mean ± SE; Asterisks indicate significant differences between spirulina and placebo groups in each category (P< 0.05 by Student's t-test)
[2)] BMI: body mass index
[3)] WHR: waist-to-hip ratio
[4)] TSF: triceps skinfold thickness
[5)] SBP: systolic blood pressure
[6)] DBP: diastolic blood pressure

Table 2. Plasma lipid profiles of the subjects during the intervention period

	Non-obese (BMI < 25 kg/m^2)					Obese (BMI ≥ 25 kg/m^2)				
	Spirulina (n = 25)		Placebo (n = 20)		P-value[2)]	Spirulina (n = 16)		Placebo (n = 17)		P-value
	Baseline	4 mo	Baseline	4 mo		Baseline	4 mo	Baseline	4 mo	
Total-cholesterol (mg/dl)	191.1 ± 6.9[1)]	179.2 ± 7.6*	196.0 ± 8.3	198.5 ± 9.4	0.049	186.5 ± 10.3	180.3 ± 8.0	196.8 ± 9.7	195.5 ± 10.6	0.8714
LDL-cholesterol (mg/dl)	120.5 ± 6.6	109.9 ± 7.4*	123.7 ± 9.2	128.2 ± 9.9	0.1250	110.1 ± 10.7	108.4 ± 9.5	129.5 ± 7.7	121.9 ± 11.0	0.2979
HDL-cholesterol (mg/dl)	51.5 ± 3.1	50.8 ± 2.7	53.7 ± 4.2	52.9 ± 3.6	0.9720	45.0 ± 4.1	40.7 ± 1.7	43.8 ± 2.9	47.9 ± 4.1	0.0881
LDL/HDL Ratio	2.51 ± 0.20	2.34 ± 0.22	2.66 ± 0.30	2.72 ± 0.27	0.3901	2.68 ± 0.26	2.73 ± 0.26	3.07 ± 0.22	2.76 ± 0.25	0.1623
Triglyceride (mg/dl)	95.3 ± 13.6	92.1 ± 9.6	92.4 ± 9.2	86.5 ± 7.5	0.8506	157.3 ± 22.3	155.5 ± 25.4	117.5 ± 15.6	128.0 ± 20.6	0.6517
AI[3)]	2.92 ± 0.21	2.74 ± 0.25	3.04 ± 0.31	3.07 ± 0.29	0.4737	3.44 ± 0.30	3.55 ± 0.27	3.63 ± 0.24	3.33 ± 0.26	0.1394

[1)] Mean ± SE; Asterisks indicate significantly different by paired t-test values between baseline and 4 months in the same group by supplement (* P< 0.05)
[2)] Pr > F value by repeated measures ANOVA for time*treatment based on BMI group
[3)] AI : atherogenic index

sugar and triglyceride level were higher in the spirulina group compared with the placebo group (P < 0.05). In the obese group, total energy intake and carbohydrate intake were higher in the placebo group compared with the spirulina group (P < 0.05).

Effects of spirulina on the lipid profiles of the subjects

In the non-obese group, spirulina supplementation showed a significant lowering effect on plasma concentration of total cholesterol and LDL-cholesterol, while no changes were observed in the placebo group. In addition, spirulina supplementation showed a significant lowering effect on plasma total cholesterol by repeated test for treatment (time × treatment interaction, P < 0.05). However, in the obese group, after 4 months of intervention, no significant changes were observed in the plasma concentrations of cholesterol and triglyceride (Table 2).

The effect of spirulina on the immune variables of the subjects

In non-obese subjects, spirulina supplementation resulted in a significant rise (time × treatment interaction, P < 0.01) in IL-2

Table 3. Immune variables of the subjects during the intervention period

	Non-obese (BMI < 25 kg/m^2)					Obese (BMI ≥ 25 kg/m^2)				
	Spirulina (n = 25)		Placebo (n = 20)		*P*-value[2]	Spirulina (n = 16)		Placebo (n = 17)		*P*-value
	Baseline	4 mo	Baseline	4 mo		Baseline	4 mo	Baseline	4 mo	
IL-2(pg/ml)	9.17 ± 0.23[1]	14.13 ± 0.33**	12.36 ± 0.62	13.38 ± 0.27	0.0012	9.78 ± 0.19	13.05 ± 0.17**	11.74 ± 0.64	12.92 ± 0.25	0.0173
IL-6(pg/ml)	1.74 ± 0.61	1.38 ± 0.39	1.19 ± 0.31	2.50 ± 0.80*	0.0535	2.33 ± 1.39	2.67 ± 1.38	1.40 ± 0.43	1.02 ± 0.23	0.3046
IL-2/IL-6	13.58 ± 1.56	21.21 ± 2.57**	20.02 ± 3.03	16.30 ± 2.77	0.0084	12.39 ± 1.63	16.15 ± 2.17*	17.23 ± 2.28	18.72 ± 2.30	0.3124
TNF-α(pg/ml)	1.87 ± 0.41	1.12 ± 0.14	2.21 ± 0.82	0.99 ± 0.04	0.6020	2.15 ± 1.52	1.06 ± 0.05	2.46 ± 0.93	1.08 ± 0.08	0.3293

[1] Mean ± SE; Asterisks indicate significant differences after supplmention in each intervention gruoup (* P< 0.05, ** P< 0.01 by Paired t-test)
[2] Pr > F value by repeated measures ANOVA for time*treatment based on BMI group

Table 4. Antioxidant variables of the subjects during the intervention period

	Non-obese (BMI < 25 kg/m^2)					Obese (BMI ≥ 25 kg/m^2)				
	Spirulina (n = 25)		Placebo (n = 20)		*P*-value[3]	Spirulina (n = 16)		Placebo (n = 17)		*P*-value
	Baseline	4 mo	Baseline	4 mo		Baseline	4 mo	Baseline	4 mo	
TAS(nmol/L)	1.60 ± 0.10[1]	2.09 ± 0.17*	1.70 ± 0.12	2.15 ± 0.18	0.8725	1.68 ± 0.15	2.14 ± 0.20	1.56 ± 0.09	2.09 ± 0.21*	0.2362
TBARS(nmol/mL)	7.12 ± 0.38	5.37 ± 0.45**	6.30 ± 0.38	6.02 ± 0.29	0.0444	7.40 ± 0.70	6.18 ± 0.62	6.94 ± 0.64	5.89 ± 0.38	0.8981

[1] Mean ± SE; Asterisks indicate significant differences after supplmention in each intervention gruoup (* P< 0.05, ** P< 0.01 by Paired t-test)
[2] Pr > F value by repeated measures ANOVA for time*treatment based on BMI group

concentration, and a significant increment (time × treatment interaction, $P < 0.01$) in IL-2/IL-6 ratio (Table 3). In the obese group, like in non-obese subjects, the level of IL-2 was significantly increased after 4 months of spirulina supplementation. However, the impact was greater in the non-obese group than in the obese group. Specifically, in the obese group, IL-2 increased by 33.4% and IL6 decreased by 14.6%, while IL-2 increased by 54.1% and IL6 decreased by 20.7% in the non-obese group. For TNF-α, no effects of supplementation with either placebo or spirulina were observed.

The effect of spirulina on the antioxidant status of the subjects

As shown in Table 4, the TAS level was significantly increased in the non-obese group after spirulina supplementation and in the obese group after placebo supplementation. No significant time-by-treatment intervention for TAS was observed in either group. TBARS level was decreased from 7.12 nmol/ml to 5.37 nmol/ml in the non-obese group after spirulina supplementation. A significant time-by-treatment intervention for TBARS was observed in the non-obese group ($P < 0.05$).

DISCUSSION

Functional foods are thought to provide benefits beyond basic nutrition and may play a role in reducing or minimizing the risk of certain diseases and other health conditions. Traditionally, functional food or dietary guidelines were provided to the general population. However, these general products or guidelines cannot support individual health needs and functional effects due to ethnic, genetic, and physiological differences.

Spirulina was believed to be associated with anti-aging, anti-cancer, dyslipidemia, hypertension, and diabetes [25-28]. These claims come from its lipid-lowering effects, immune-enhancing effects, and antioxidant capacity. In our previous studies, we also confirmed that spirulina supplementation had a positive effect on lipid profiles, immune response, and antioxidant capacity [14]. However, we found the possibility that effects of spirulina show different aspects depending on the subjects' obesity. Therefore, in this study, we divided the subjects into two groups, non-obese and obese group, and examined the effects of spirulina supplementation on lipid-lowering effect, immunomodulatory, and antioxidative capacity of each group. The results showed that spirulina supplementation reduced plasma concentration of LDL-cholesterol in the non-obese group, however this effect was not observed in obese subjects. The non-obese group showed a higher increase in IL-2 level e by spirulina supplementation compared with the obese group. Also, by spirulina supplementation, TAS showed a greater increase, and TBARS showed a greater decrease in non-obese subjects than in obese subjects. From these results, we could confirm that the intervention effect of spirulina differs according to obesity.

Obesity is associated with systemic inflammation and impaired immunity. In a study of treatment response in obese subjects, Weber *et al*. [29] first reported that obesity may be a predictor of poor antibody response. In obese individuals, visceral adipose tissue is the major source of proinflammatory cytokines [30]. Osborn *et al*. [31] reported that adipocytes secrete TNF-α, IL-6, IL-12, IL-1β, and monocyte chemotactic protein-1(MCP-1) in large amounts. Obesity can also induce oxidative stress in adipocytes via production of reactive oxygen species (ROS) by mitochondria which can be increased in response to a high-fat diet [32,33]. In a human study, Keaney *et al*. [15] clearly demonstrated the association between oxidative stress and obesity by monitoring urinary isoprostanes, and Paich *et al*. [34] reported that overweight and obese adult humans have a defective cellular immune response to pandemic H1N1 influenza A virus. Scott *et al*. [35] reported that one important factor correlating with decreased vaccine-induced immune response is obesity, which may affect adaptive immune responses. IL-2, an anti-inflammatory cytokine, is an essential regulator of chronic inflammatory responses [36]. As the concentration of plasma IL-2 decreases according to aging, raising plasma IL-2

levels is important for proper immune regulation in elderly people [37]. In our study, the effect of spirulina on increasing IL-2 was greater in the non-obese group than in the obese group. Also, in this study, lipid-lowering effect and antioxidant capacity appeared to be low in the obese group, indicating that health promotion effect of spirulina supplement can be reduced in obese people. It is uncertain whether this is due to pathological and immunological changes caused by the obesity itself, or to relatively low spirulina supplementation to the weights of obese people. It is clear that obese people require different doses of spirulina supplementation compared with the non-obese group. It is not only for spirulina, but other dietary supplements may require different doses in obese people. In fact, β-cell function tended to be greater following L-carnitine supplement in the lean group only [38]. And curcuminoids supplementation, which have potentially important functional qualities including anti-inflammatory and antioxidant properties, resulted in no significant change in serum concentrations of oxidative markers anti-Hsp27 and anti-oxidized LDL in obese individuals [39].

Intake guidelines for functional food, including spirulina, are established to be appropriate for the general public. The result of this study shows that intake effect of functional food differs according to personal characteristics including obesity, therefore the guidelines for these foods should be diversified for individuals.

In conclusion, this randomized double-blind, placebo-controlled study demonstrated that 4 months supplementation of spirulina significantly decreased the plasma level of cholesterol and LDL-cholesterol, increased the plasma level of IL-2, and increased the antioxidant capacity, and these effects of spirulina supplementation were ameliorated by obesity. Our results suggest that obesity may influence the effect of spirulina supplementation. However the mechanism between obesity and spirulina supplement response it is not yet clear. It can be assumed, as described above, obesity itself may impair immunity and can also induce oxidative stress. Further studies are needed to determine the mechanism of spirulina on lipid profiles, immune variables, and antioxidant capacity based on BMI.

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