Genetic Variation in MCP-1 Is Associated with the Efficacy of Spirulina Supplementation on Antioxidant Levels in Elderly Korean: a Randomized Double Blind Trial

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ABSTRACT

Spirulina is a known functional food related to antioxidant capacity. Gene variation may impact functional food supplementary effects. The current study investigated the interaction of monocyte chemoattractant protein-1 (MCP-1)-2518 polymorphism with spirulina supplements on antioxidant capacity in Korean elderly (n = 78). The subjects consumed 8 g/day either spirulina or placebo for 4 months by using a randomized, double blind, and placebo-controlled design. Antioxidant level, body composition, and dietary intake were measured twice (baseline vs. week 16). Plasma total antioxidant status (TAS), thiobarbituric acid reactive substance (TBARS), vitamin A, vitamin C, vitamin E, and folate were analyzed as antioxidant markers. For the subject with A allele, the plasma level of antioxidant nutrient such as vitamin A, and folate was significantly higher in spirulina group than placebo. The plasma TBARS level was significantly lower in spirulina group than placebo. For the subject with G/G genotype, among the antioxidant nutrients, only folate level was significantly higher in spirulina group than placebo. The plasma TAS level was significantly higher in spirulina group with G/G genotype. The effect of treatment × time was only found in plasma level of folate and TBARS in A/A genotype. In conclusion, MCP-1 genotype is associated with antioxidant efficacy in response to spirulina supplementation. These results may be useful for personalized nutritional recommendation to improve antioxidant capacity in healthy Korean elderly.

Keywords: Genetic variation; Monocyte chemoattractant protein 1; Spirulina; Antioxidants; TBARS

INTRODUCTION

Monocyte chemoattractant protein-1 (MCP-1) involved in cardiovascular disease, 1,6 MCP-1 promotes the transmigration and emigration of circulating monocytes into tissues, and exerts various effects on monocytes, including the induction of superoxide anions and expression of various proinflammatory genes. 1,2 MCP-1 is expressed at high levels in atherosclerotic plaques 1 and the level of MCP-1 has also been shown to increase in patients with myocardial infarction, unstable angina, and venous thrombosis, and is considered to involve in the development and progression of cardiovascular disease. 1,6

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Oxidative damage and antioxidant protection are also associated with cardiovascular disease. Under oxidative stress, oxidative modifications of low-density lipoprotein (LDL) and lipid peroxidation take place. And also, there is endothelial dysfunction, monocyte adhesion, macrophage uptake of lipoprotein and inflammatory process. Reactive oxygen species are known to stimulate the production of inflammatory mediators like interleukin (IL)-8 and MCP-1. Therefore, antioxidant food can ameliorate the inflammatory process. Aronia melanocarpa which constitute important phytochemicals including proanthocyanidins, anthocyanins, and hydroxycinnamic acids reduces the MCP-1 level and indicated inhibition of neutrophil infiltration in the injured site. Ko and Kim reported diet composition and long-term exercise training for 8 weeks decreased inflammation via antioxidant lowering effect. In the animal experiment, it was confirmed that the MCP-1 level decreased when the antioxidant gene such as hypoxia inducible factor-1 and extra cellular signal regulate kinase was suppressed.

Single nucleotide polymorphism (SNP) in the MCP-1 promoter region have been identified and shown to affect transcription of the gene. The MCP-1 gene A-2518G polymorphism confers an increased risk of vascular complications in type 2 diabetes mellitus patients. The 2518A/G polymorphism in the MCP-1 gene is associated with cancer, tuberculosis risk, and mood disorder. Genetic variation could modulate the efficacy of functional food supplementation. Mohseni reported vitamin D receptor gene polymorphism related the efficacy of vitamin D supplementation on inflammatory marker and total antioxidant lowering effect. Borel and Desmarchelier described that genetic variation is associated with postprandial blood vitamin A concentration and vitamin A bioavailability. The effect of vitamin E supplementation on blood haptoglobin was different by Haptoglobin genotype in Singapore diabetes patients. For MCP-1 gene variation, few papers have studied the effect of dietary supplements according to the MCP-1 gene polymorphism. In our previous study, the differences in cytokine and MCP-1 according to the MCP-1 gene polymorphism was drawn after spirulina supplementation.

Spirulina provides an adequate amount of a spectrum of carotenoids pigments, especially β-carotene and zeaxanthin which components are well known to have antioxidant properties. Spirulina is a microscopic and filamentous cyanobacterium (blue-green alga) that has a long history of use as food which is a rich source of vitamins, especially vitamin B12 and provitamin A (β-carotene), minerals, carotenoids, zeaxanthin, phycocyanins. All components of spirulina are well known to have antioxidant properties. Many studies have reported that spirulina is overwhelming in the areas of cholesterol-regulatory properties, immunomodulation, antioxidant capacity, and anti-cancer. In human study, spirulina maxima decrease endothelial damage and oxidative stress indicators in patients with systemic arterial hypertension. And spirulina supplementation considerably improved total antioxidant status (TAS) compared to placebo-treated individuals. However, there are few studies on the interaction of spirulina with genetic variants.

MCP-1 is significantly increased under conditions of oxidative stress, which leads to cardiovascular disease. The supplement that can inhibit oxidative damage could decrease the expression of MCP-1, and it may interfere with cardiovascular disease progression. From here, it is important to consider that MCP-1 expression is affected by the MCP-1 genotype. Therefore, this study was performed to examine the responsiveness of food intervention according to MCP-1 genotype considering the limited evidence available on the relationships between the individual response to spirulina induced antioxidant effect and MCP-1 gene variation.
METHODS

Subjects and experimental design
The healthy subjects for intervention study were recruited through an advertisement in local newspaper (2005.12–2006.6). The volunteers were first interviewed by telephone for screening. The exclusion criteria for subject was current user of vitamin supplements, current drug-user of inflammatory disease (e.g., Crohn disease, rheumatoid arthritis), dyslipidaemia, and hypertension, concurrent or recent participant in other intervention study. Finally, 78 subjects (male 43, female 35) were enrolled in this study. The present study protocol was reviewed and approved by the Institutional Review Board of Ewha Women’s University (ECT 109-02-01). Informed consent was submitted by all subjects when they enrolled.

This study was a 16-week randomized, double-blind, placebo-controlled trial of spirulina supplementation in elderly Korean. The subjects consumed either the spirulina or placebo, 8 g in a day. During the intervention period, subjects were asked to keep usual diet, and prohibited to take any functional food or supplements. Both spirulina (100% spirulina) and placebo (100% starch) were provided by Earth Spirulina group (Seoul, Korea). Compliance was checked by calling the subjects twice a week.

Dietary intake and anthropometric measures
The food consumption was assessed using 24-hour recall method. Dietary intake data was analyzed by using CAN-Pro 4.0 software (The Korean Nutrition Society, Seoul, Korea). Height was measured using an anthropometer (Seca 213; Seca Inc., Birmingham, UK). Body weight and composition were measured using INBODY 2.0 (Biospace Co., Seoul, Korea) with subjects wearing light clothing.

Blood collecting and measurement
Blood samples were taken on baseline and 16th week of the study period. Serum and plasma samples were separated by centrifuge with 2,000 rpm for 25 minutes and the samples were stored at −80°C freezer until measurement. Total cholesterol and triglyceride level were measured by using autoanalyzer (Ekachem DTSC module; Johnson & Johnson, New Brunswick, NJ, USA). High-density lipoprotein cholesterol level was determined by autoanalyzer after treating UC infranatant with phosphotungstic acid-Mg. LDL-cholesterol (LDL-C) was calculated by Friedewald method. Plasma MCP-1 concentrations were determined by enzyme linked immunosorbent assay (ELISA) technique (Quantikine; R&D Systems Inc., Minneapolis, MN, USA) reading ELISA reader (Spectra Max 340; Molecular Devices, San Jose, CA, USA).

Plasma level of vitamin A, C, E, and folate
Plasma level of retinol and α-tocopherol were determined using reversed-phase high performance liquid chromatography (HPLC). Briefly, the methanol was added to precipitate protein, and n-haxane was added to extract lipids. The mixed solution was centrifuged at 3,000 rpm for 15 minutes at room temperature. After centrifuging, the hexane layer was moved in brown vial, and volatilized by N₂ gas using a vacuum evaporator. The remainder was dissolved in 1 mL of eluent (dichloromethane:methanol = 15:85) and filtered using a 0.45 μm filter (NYLON 66 SYRINGE FILTER; Whatman, Florham Park, NJ, USA). Alpha-tocopherol and retinol were measured using HPLC (Waters 2690 Separation Module; Waters, Milford, MA, USA) equipped with UV detector (YoungLin Co. Ltd., Anyang, Korea) using a XTerra™ RP18 column (5 μm; 4.6 × 150 mm; Waters). The absorbance of samples was quantified using...
the Autochro-WIN program (version 2.0 plus; YoungLin Instruments Co., Anyang, Korea). All extraction procedures were performed under dark conditions. Mobile phase consisted of methanol and dichloromethane (85:15, v/v). The flow rate was 0.8 mL/min. The detection wavelengths were set to 295 nm for Alpha-tocopherol and to 325 nm for retinol.

Plasma vitamin C was determined by colorimetric vitamin C assay kit (Immundiagnostik AG, Bensheim, Germany). Serum folate level was measured using 125I-folic acid and 57Co-Vitamin B12 dualcount SPNB (solid phase no boil) radioassay kit (Diagnostic Products Co., Los Angeles, CA, USA). The final concentration was determined by dualy-counter (CobraII, auto-gamma; Mirion Technologies, Inc., San Ramon, CA, USA).

**Assay of antioxidant capacity**

Plasma concentration of thiobarbituric acid reactive substance (TBARS) concentration was determined using Yagi method and TBARS concentration was determined with luminescence spectrometer (LS50; Perkin Elmer, Waltham, MA, USA) at excitation 515nm, emission 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. TAS of plasma sample was measured using a commercial TAS kit (Randox Laboratories Ltd, Crumlin, UK).

**DNA extraction and analysis of the MCP-1 polymorphism**

All subjects were genotyped for MCP-1 polymorphism (dbSNP: rs1024611). The DNA was extracted from whole blood (QIAamp DNA Blood Kit; Qiagen, Hilden, Germany), and a 649 bp segment of MCP-1 gene including polymorphism sites was amplified by the polymerase chain reaction (forward primer: 5′-AGT CCA ACC AAG GTT TGT GC-3′ and the reverse primer 5′-TCA TGC TTC GGG TTT TCT CT-3′). The presence of MCP-1 polymorphism was determined by restriction fragment length polymorphism assay using PvuII enzyme.

**Statistical analysis**

Analysis was conducted using SPSS Statistics version 24. Values are shown as the mean and standard error. The results were presented for placebo and spirulina group by genotype. Paired t-test was used to analyze mean differences for all measured parameters between pretest and posttest. To examine the effect of treatment, repeated measured analysis of variance test was conducted.

**RESULTS**

**MCP-1 genotype and self-reported health behavior**

Seventy-eight healthy volunteers were enrolled in this intervention study and were divided into placebo (n = 37) and spirulina groups (n = 41) by double-blind and randomization. The subjects’ age was 65.9 years, there were no significant differences in age, sex, and genotype distribution between 2 groups within same genotypes (Table 1). All participants were in good health based on the medical history and medication. More than 90% of the subjects were non-smokers, and 60% of subjects were regular exercisers. The health status and health behavior were not significantly different in both groups within same genotype and among the genotype.
Baseline characteristics

Baseline characteristics by supplementation group were shown in Table 2. The anthropometric parameters were not different between 2 groups within same genotype. No differences were observed in the MCP-1 level, lipid profiles, and blood pressure by the groups.

Table 2. Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Placebo</th>
<th>Spirulina</th>
<th>Placebo</th>
<th>Spirulina</th>
<th>Placebo</th>
<th>Spirulina</th>
<th>Placebo</th>
<th>Spirulina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>61.8 ± 1.44</td>
<td>64.3 ± 1.87</td>
<td>65.3 ± 4.65</td>
<td>69.0 ± 4.25</td>
<td>61.6 ± 1.96</td>
<td>64.2 ± 2.53</td>
<td>61.1 ± 2.54</td>
<td>58.3 ± 1.41</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.3 ± 0.41</td>
<td>24.6 ± 0.49</td>
<td>26.3 ± 2.15</td>
<td>25.3 ± 1.21</td>
<td>24.2 ± 0.56</td>
<td>24.9 ± 0.62</td>
<td>23.9 ± 0.48</td>
<td>22.6 ± 0.48</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>85.1 ± 1.27</td>
<td>86.9 ± 1.57</td>
<td>88.7 ± 5.60</td>
<td>90.6 ± 3.70</td>
<td>84.7 ± 1.83</td>
<td>86.2 ± 2.13</td>
<td>84.5 ± 1.68</td>
<td>84.0 ± 1.49</td>
</tr>
<tr>
<td>WHR (cm)</td>
<td>0.86 ± 0.01</td>
<td>0.87 ± 0.01</td>
<td>0.89 ± 0.15</td>
<td>0.87 ± 0.01</td>
<td>0.86 ± 0.01</td>
<td>0.86 ± 0.01</td>
<td>0.86 ± 0.01</td>
<td>0.87 ± 0.01</td>
</tr>
<tr>
<td>TSF (mm)</td>
<td>23.7 ± 1.47</td>
<td>25.9 ± 1.42</td>
<td>26.0 ± 7.26</td>
<td>24.8 ± 3.57</td>
<td>23.6 ± 2.08</td>
<td>26.3 ± 1.78</td>
<td>23.2 ± 1.94</td>
<td>26.1 ± 3.14</td>
</tr>
<tr>
<td>Bodyfat (%)</td>
<td>31.4 ± 3.59</td>
<td>25.5 ± 1.07</td>
<td>28.8 ± 1.07</td>
<td>30.3 ± 1.21</td>
<td>29.9 ± 1.18</td>
<td>25.7 ± 2.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>100.9 ± 2.9</td>
<td>105.1 ± 2.1</td>
<td>105.8 ± 15.5</td>
<td>97.0 ± 4.3</td>
<td>98.1 ± 3.1</td>
<td>108.8 ± 2.8</td>
<td>104.0 ± 5.6</td>
<td>104.1 ± 3.8</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>186.4 ± 6.2</td>
<td>189.4 ± 5.79</td>
<td>186.5 ± 27.6</td>
<td>181.6 ± 9.4</td>
<td>195.8 ± 7.9</td>
<td>193.6 ± 8.2</td>
<td>200.7 ± 10.9</td>
<td>185.8 ± 14.4</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>126.3 ± 6.9</td>
<td>116.5 ± 5.83</td>
<td>115.1 ± 20.8</td>
<td>116.3 ± 9.7</td>
<td>124.6 ± 9.2</td>
<td>117.6 ± 8.5</td>
<td>132.9 ± 13.0</td>
<td>113.3 ± 13.1</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>49.3 ± 2.77</td>
<td>48.9 ± 2.48</td>
<td>45.0 ± 5.2</td>
<td>43.9 ± 3.3</td>
<td>50.8 ± 4.4</td>
<td>48.4 ± 3.3</td>
<td>48.3 ± 3.77</td>
<td>58.3 ± 7.1</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>103.6 ± 8.7</td>
<td>119.5 ± 12.8</td>
<td>132.3 ± 45.2</td>
<td>107.2 ± 17.1</td>
<td>101.9 ± 11.2</td>
<td>138.4 ± 19.7</td>
<td>97.0 ± 12.5</td>
<td>72.3 ± 7.43</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>2.84 ± 0.19</td>
<td>2.58 ± 0.14</td>
<td>2.58 ± 0.40</td>
<td>2.75 ± 0.28</td>
<td>2.79 ± 0.24</td>
<td>2.65 ± 0.22</td>
<td>3.03 ± 0.41</td>
<td>2.08 ± 0.31</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>126.4 ± 2.81</td>
<td>135.6 ± 2.38</td>
<td>143.1 ± 12.7</td>
<td>141.8 ± 4.02</td>
<td>131.9 ± 3.6</td>
<td>133.2 ± 3.2</td>
<td>141.1 ± 4.2</td>
<td>135.3 ± 6.1</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>84.9 ± 1.37</td>
<td>83.2 ± 1.57</td>
<td>89.3 ± 3.7</td>
<td>87.4 ± 3.7</td>
<td>83.4 ± 1.9</td>
<td>81.4 ± 2.1</td>
<td>86.1 ± 2.3</td>
<td>82.2 ± 2.2</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>1,593.0 ± 89.3</td>
<td>1,503.9 ± 55.6</td>
<td>1,737.6 ± 121.2</td>
<td>1,662.7 ± 148.4</td>
<td>1,549.4 ± 122.4</td>
<td>1,467.6 ± 56.5</td>
<td>1,492.6 ± 122.2</td>
<td>1,401.5 ± 151.9</td>
</tr>
<tr>
<td>Vitamin A intake (µg RE)</td>
<td>447.0 ± 43.5</td>
<td>384.4 ± 29.9</td>
<td>381.5 ± 120.8</td>
<td>341.1 ± 42.7</td>
<td>473.3 ± 62.3</td>
<td>385.3 ± 46.5</td>
<td>349.1 ± 58.3</td>
<td>414.8 ± 64.8</td>
</tr>
<tr>
<td>Vitamin C intake (mg)</td>
<td>123.1 ± 17.5</td>
<td>113.2 ± 11.3</td>
<td>99.6 ± 51.9</td>
<td>127.3 ± 22.1</td>
<td>146.2 ± 29.3</td>
<td>108.1 ± 14.0</td>
<td>94.7 ± 12.8</td>
<td>110.3 ± 36.8</td>
</tr>
<tr>
<td>Vitamin E intake (mg TE)</td>
<td>8.77 ± 0.92</td>
<td>7.52 ± 0.79</td>
<td>9.45 ± 2.8</td>
<td>7.42 ± 1.5</td>
<td>7.56 ± 1.01</td>
<td>8.2 ± 1.0</td>
<td>9.3 ± 1.7</td>
<td>6.94 ± 1.2</td>
</tr>
<tr>
<td>Folate intake (µg DFE)</td>
<td>246.6 ± 24.2</td>
<td>222.9 ± 18.8</td>
<td>285.4 ± 43.7</td>
<td>236.9 ± 37.6</td>
<td>241.9 ± 28.5</td>
<td>233.0 ± 27.4</td>
<td>212.6 ± 19.4</td>
<td>198.8 ± 13.2</td>
</tr>
</tbody>
</table>

Data are shown as mean ± standard error or number (%).

BMI = body mass index, WHR = waist-to-hip ratio, TSF = triceps skinfolds thickness, LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, SBP = systolic blood pressure, DBP = diastolic blood pressure.
Dietary intakes were not different according to supplement group. The intake of total energy, vitamin A, C, E, and folate related to antioxidant status were similar between the groups.

**Dietary intakes**
The subject’s dietary intake during the intervention period may confuse the effect of the test substance. Therefore, by examining the amount of dietary intake before and after the supplementation, it was attempted to exclude confounding factors from supplement efficacy.

The total food intake was not changed in the subjects with A/A or G/G genotype during intervention period. There was no difference in daily nutrients intake in the subjects with A/A or G/G genotype after supplementation either spirulina or placebo (Table 3). In the G/A genotype, daily intakes of energy, Ca, vitamin A, carbohydrate, phosphate, and vitamin B2 were changed after intervention in both groups. However, significant time-by-treatment interaction was not observed.

**Plasma level of vitamin A, C, E, and folate**
The response for antioxidant nutrients was shown in Table 4. In all subjects, plasma level of retinol was decreased, and folate level was increased after intervention period for both groups. There were no significant differences in the plasma level of retinol and α-tocopherol level during intervention period in each genotype. In the A/A genotype, the plasma level of vitamin C and folate were increased after spirulina supplement, from 9.97 pg/mL to 11.5 pg/mL, and from 7.63 nmol/L to 17.6 nmol/L, respectively. In the G/A and G/G genotype, the folate level was significantly increased. The effect of treatment was found in plasma level of folate (time × treatment interaction $P < 0.05$) in all subject and in A/A genotype group.

**Antioxidants capacity**
The response for antioxidant status was shown in Table 5. In all subjects, TBARS level was decreased and TAS level was significantly increased after spirulina supplementation ($P < 0.05$). However, no significance was found in the effect of treatment × time. In the A/A

### Table 3. Background intake during intervention according to monocyte chemoattractant protein-1 genotype

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>All</th>
<th>A/A</th>
<th>G/A</th>
<th>G/G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>0.5143a</td>
<td>0.1806</td>
<td>0.5379</td>
<td>0.1297</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0.6778</td>
<td>0.5189</td>
<td>0.2122</td>
<td>0.0537</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.9618</td>
<td>0.5037</td>
<td>0.7229</td>
<td>0.1615</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>0.2917</td>
<td>0.7179</td>
<td>0.2853</td>
<td>0.4801</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>0.3075</td>
<td>0.1774</td>
<td>0.6721</td>
<td>0.7208</td>
</tr>
<tr>
<td>Ca (mg)</td>
<td>0.8989</td>
<td>0.6321</td>
<td>0.9357</td>
<td>0.2901</td>
</tr>
<tr>
<td>P (mg)</td>
<td>0.5857</td>
<td>0.3122</td>
<td>0.2708</td>
<td>0.1547</td>
</tr>
<tr>
<td>Fe (mg)</td>
<td>0.1428</td>
<td>0.5213</td>
<td>0.3169</td>
<td>0.1076</td>
</tr>
<tr>
<td>Zn (mg)</td>
<td>0.3514</td>
<td>0.7477</td>
<td>0.2364</td>
<td>0.1820</td>
</tr>
<tr>
<td>Vitamin A (µg RE)</td>
<td>0.2707</td>
<td>0.3547</td>
<td>0.2284</td>
<td>0.4980</td>
</tr>
<tr>
<td>Vitamin B1 (mg)</td>
<td>0.9320</td>
<td>0.3719</td>
<td>0.6827</td>
<td>0.3526</td>
</tr>
<tr>
<td>Vitamin B2 (mg)</td>
<td>0.7013</td>
<td>0.2970</td>
<td>0.2213</td>
<td>0.1252</td>
</tr>
<tr>
<td>Vitamin B3 (mg)</td>
<td>0.2165</td>
<td>0.1773</td>
<td>0.2417</td>
<td>0.6351</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>0.8290</td>
<td>0.9972</td>
<td>0.9227</td>
<td>0.2794</td>
</tr>
<tr>
<td>Vitamin E (mg TE)</td>
<td>0.4986</td>
<td>0.1837</td>
<td>0.4183</td>
<td>0.7231</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>0.3379</td>
<td>0.8380</td>
<td>0.1587</td>
<td>0.4626</td>
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<tr>
<td>Folic acid (µg DFE)</td>
<td>0.4613</td>
<td>0.3155</td>
<td>0.7897</td>
<td>0.5197</td>
</tr>
<tr>
<td>% Carbohydrateb</td>
<td>0.7476</td>
<td>0.6743</td>
<td>0.3138</td>
<td>0.0617</td>
</tr>
<tr>
<td>% Protein</td>
<td>0.7690</td>
<td>0.6360</td>
<td>0.1677</td>
<td>0.0476</td>
</tr>
<tr>
<td>% Fat</td>
<td>0.5214</td>
<td>0.9929</td>
<td>0.5426</td>
<td>0.3074</td>
</tr>
</tbody>
</table>

*P > F: repeated measure analysis of variance; bThe percentage contribution of the 3 macronutrients (carbohydrate, protein, and fat) to the total daily energy intake.
genotype, the TBARS level was significantly increased in placebo group and decreased in spirulina group, from 6.27 nmol/L to 7.21 nmol/L for placebo, from 7.03 nmol/L to 5.27 nmol/L for spirulina. In the G/A genotype, TBARS and TAS level was not changed after supplementation with spirulina. In the subject with G/G genotype, TAS level was significantly increased in spirulina group, from 1.60 nmol/L to 2.25 nmol/L. The TBARS level tended to decrease in spirulina group. The effect of treatment was found in plasma level of TBARS (time × treatment interaction \( P < 0.05 \)) in subject with A/A genotype.

**DISCUSSION**

Our study indicated that genotype-diet interaction was observed for MCP-1 variation in the response of spirulina supplement on the antioxidant capacity. Spirulina is reported to be effective to improve blood lipid profiles, to enhance immune capacity, and to reduce oxidative stress.23-28 For the antioxidant properties of spirulina, Benedetti et al.33 reported that spirulina inhibited lipid peroxidation more significantly (65% inhibition) than the other antioxidants like \( \alpha \)-tocopherol (35%), butylated hydroxytoluene (45%), and \( \beta \)-carotene (48%) in vitro. In animal study,34 plasma antioxidant capacity was higher in spirulina treatment group than control group.

In terms of MCP-1 polymorphism, many data from studies have shown that A/A genotype was wild-type and G/G homozygotes frequency was increased among cardiovascular disease's
patients in Europe and America. A few studies conducted with Korean population reported that the G/G genotype, not A/A genotype, was wild-type genotype. Kim et al. informed that the frequency of A/A, G/A, and G/G genotype among healthy Korean was 20%, 42%, 38%, respectively, and Pae et al. also found that A/A genotype was 9.6% in healthy Korean population. Hwang et al. reported that A/A genotype and G/G genotype were 16% and 46%, respectively, in the healthy Koreans. The frequency of the G-allele is about 2 times higher in Koreans than in Caucasians and Afro-Americans. These studies suggest that there is racial difference in the distribution of MCP-1 polymorphism. In Korean, MCP-1-2518A allele is minor allele and minor allele was associated with plasma MCP-1 level. MCP-1-increasing allele which is more sensitive to risk factors and are more prone to development of cardiovascular disease.

Genetic background may interact with supplementation and influence the effect of the supplement. Perez-Martinez et al. reported gene-nutrient interactions on phosphoenolpyruvate carboxykinase and insulin sensitivity. Among subjects with n-3 polyunsaturated fatty acid (PUFA) levels above the population median, carriers of the C/C genotype exhibited lower plasma concentrations of fasting insulin and homeostasis model assessment of insulin resistance as compared with C/C carriers with n-3 PUFA below the median. And in another study, glucokinase regulatory protein genetic variant interacts with omega-3 PUFA in metabolic syndrome. In addition to longitudinal studies, gene variation effect in response to supplementation have shown among clinical trials, with response ranging from better than average to nonresponse or even adverse response. Oatmeal has been widely known for cholesterol-lowering effect. However, Ye et al. reported that response of serum LDL-C to oatmeal consumption depends on cytochrome P450 family 7 subfamily A member 1 gene rs3808607 (CYP7A1) genotype in Chinese. MacKay et al. also demonstrated CYP7A1 and apolipoprotein E (APOE) isoform are associated with the extent of reduction in circulating LDL-C in response to plant sterol consumption. More specifically, CYP7A1 T/T homozygotes showed no LDL-C lowering effect, whereas the presence of the G-allele associated with LDL-C response in a dose dependent fashion. Moreover, genoset CYP7A1 T/T/APOE ε3 was associated with nonresponsiveness for plant sterol consumption. These results could serve as personalized markers to dietary supplement consumption.

Fewer studies have investigated the interaction between MCP-1 genotype and supplementation. In our previous study, the effects of spirulina supplementation were different depending on MCP-1 genotypes. In A/A genotype, spirulina supplementation resulted in significant changes for the plasma levels of MCP-1, IL-2; MCP-1 level was decreased, and the levels of IL-2 was increased. The concanavalin A- and phytohemagglutinin-induced mitogenesis was improved after spirulina supplementation. However, other genotype showed fewer effective results. The significantly increasing effect for IL-2 level was observed in G/G genotype and increasing effect for IL-2 level was only found in G/G genotype. In this study, for the subject with A allele, the plasma level of antioxidant nutrient such as vitamin A, and folate was significantly higher in spirulina group than placebo. The plasma TAS was significantly higher and the TBARS level was significantly lower in spirulina group than placebo. For the subject with G/G genotype, among the antioxidant nutrients, only folate level was significantly higher in spirulina group than placebo. The plasma TAS level was significantly higher in spirulina group with G/G genotype. These results suggest that personalized management might be needed in different genotype. Moreover, it is important to exclude the confounding factors of the results as much as possible by examining the subjects’ basic lifestyle, eating habits, and dietary intake. In particular, dietary intake, alcohol consumption, and smoking can affect the blood antioxidant index regardless of supplementation. In this study, it was
confirmed that there was no difference in dietary intake, drinking and smoking according to genotype, and only the efficacy of supplements was examined.

One limitation of our trial was size (n = 78), which could be considered small for investigation of genetic associations. The evidence from this study is not enough to start making specific personalized nutritional recommendations based on genetic information, results from this study are valuable for further study to document the relationship between MCP-1 polymorphism and diet, and careful attention should be paid to subsequent studies to involve enough subjects for each phenotype and genotype with sufficient statistical power.

In conclusion, our data represent a beginning step in evaluating the use of common genetic variations to predict an individual’s response to supplementation. The efficacy of the spirulina supplement in reducing lipid peroxidation products was associated with MCP-1 allele, with A/A allele carriers being more responsive to spirulina supplement in reducing TBARS and increasing plasma folate level than G/G homozygous individuals. Our results demonstrate that the response of plasma antioxidant status to spirulina supplement has high interindividual variability, which is influenced by MCP-1 polymorphisms. These genetic variants could be used in the future to identify individuals who will benefit the most from spirulina intervention in terms of antioxidant capacity. The use of supplement, in the context of personalized nutritional recommendations, would greatly increase its efficacy in reducing risk factors of target disease.

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