**Effect of Chlorella Ingestion on Oxidative Stress and Fatigue Symptoms in Healthy Men**

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**Summary:** **Objective:** We examined the effects of dietary chlorella ingestion on oxidative stress and fatigue symptoms in healthy men under resting and fatigue conditions.

**Method:** We conducted a double-blind, parallel-arm controlled study. Twenty-seven healthy male volunteers (mean age, 35.4±10.4 years) were randomly divided into the chlorella and placebo groups, and received chlorella (6 g/day) and lactose as placebo (7.2 g/day), respectively, for 4 weeks. To simulate mild fatigue, subjects underwent exercise (40% of the heart rate reserve) for 30 minutes. Fatigue was measured using the visual analog scale of fatigue (F-V AS) pre- and post-exercise. Serum antioxidant capacity (AC), malondialdehyde levels, and other indices of oxidative stress were measured pre- and post-exercise. All measurements were repeated after the intervention period and the results were compared with baseline measurements.

**Results:** Under resting conditions, AC significantly increased after the intervention period in the chlorella group, but not in the placebo group. Malondialdehyde levels after the intervention period were significantly lower in the chlorella group than in the placebo group. There were no significant differences in any of the oxidative-stress indices measured pre- and post-exercise, either before or after intervention, in either group. F-VAS significantly increased after exercise at all measurement time-points in both groups, except after the intervention period in the chlorella group. Under fatigue conditions, there were no significant differences in oxidative stress indices between the groups.

**Conclusions:** Our results suggest that chlorella ingestion has the potential to relieve oxidative stress and enhance tolerance for fatigue under resting conditions.

**Key words** chlorella, oxidative stress, antioxidant capacity, fatigue, fatigue visual analog scale, exercise

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**INTRODUCTION**

The effect of mental and/or physical stress and fatigue on human health is gradually being recognized. A fiscal 2012 survey of worker health by Japan’s Ministry of Health, Labor and Welfare showed that 60.9% of workers had strong anxiety, worries, and mental and/or physical stress in their professional life, a sharp increase from the 58% reported in fiscal 2007 [1]. Further, according to a fatigue survey of approximately 20,000 workers in Yamaguchi Prefecture in 2009, more than 70% of workers experienced fatigue [2]. Collectively, these surveys indicate that workers are being exposed to excessive stress and fatigue in...
recent years, and more countermeasures are required. Although specific precipitating factors of fatigue have not been identified, oxidative stress can generate somatic symptoms, including fatigue [3]. Oxidative stress generated by physical activity may be regarded as one of the causes of fatigue [4]. Reactive oxygen and nitrogen species (RONS) are usually removed immediately from the body by antioxidants and antioxidant enzymes; however, imbalances in RONS, and depressed antioxidant ability in response to excessive stress could give rise to tissue damage resulting in fatigue [5]. Furthermore, accumulation of RONS is thought to lead to aging and various diseases including arteriosclerosis and cancer, and therefore countermeasures are important from the viewpoint of disease prevention [6-8]. Many studies have been conducted on the effect of dietary supplements such as α-lipoic acid, co-enzyme Q10, and vitamin C and E in excessive exercise-induced oxidative stress [9-11]. However, few studies have examined the effect of antioxidants on oxidative stress under fatigue conditions caused by daily work.

We focused our attention on chlorella, well documented for its safety in human use, since it contains an abundance of antioxidants. One preliminary research found that chlorella ingestion in humans enriches antioxidants such as the lutein in erythrocytes [12]. Furthermore, protective effects of chlorella against oxidative stress in the brain, and preventive effects of chlorella in cognitive decline, have been identified by animal experiments [13, 14]. However, the effect of chlorella on oxidative stress under fatigue conditions in humans has not been elucidated. The aim of this study was to examine changes in oxidative stress under fatigue conditions caused by an ordinary daily physical workload. Further, we also determined the effects of chlorella ingestion on oxidative stress under resting and fatigue conditions.

**MATERIALS AND METHODS**

**Study protocol**

We conducted a double blind, parallel-arm controlled study. The subjects were healthy male workers in Fukuoka Prefecture who were invited to participate in a public offering. We excluded subjects with a history of severe medical illness and those who were smokers or regularly used drugs and health foods, which could affect the study results. Most subjects were desk workers, whose work involved minimum physical activity. In addition, subjects were instructed to report any excessive physical activity during the intervention period. The subjects were randomly divided into chlorella or placebo groups, using the age-based permuted block method, by a person not directly involved in the study. Prior to the study, a physician explained the study contents to the study subjects and obtained written consent from them. They were instructed not to change their living conditions, and to avoid excessive exercise and alcohol consumption. Subjects in each group were administered the test diet orally for 4 weeks. Height, body weight, pulse, blood pressure, and Fatigue Visual Analog Scale (F-VAS), were measured before and after fatigue exercise load in all the study subjects. Blood samples were collected from the antecubital vein at rest (baseline) and immediately after exercise, and were used for oxidative stress indice measurement (Figure 1). Physical examination, blood collection, and fatigue exercise load were performed in a room maintained at constant temperature (approximately 24°C) and humidity (approximately 50%-55%). Blood samples were collected by nurses while other evaluations were conducted by trained staff.

All measurements were repeated after 4 weeks of intervention and the results were compared with the baseline. This study was approved by the Mii Campus Ethics Committee, Kurume University (study number: 219).

**Fig. 1.** Experimental time line.

HRR: Heart rate reserve, F-VAS: Visual analog scale of fatigue, BMI: body mass index
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EFFECT OF CHLORELLA INGESTION

Test-food and method of administration

Chlorella (*Parachlorella beijerinckii*) was cultured, powdered, and formulated into 200 mg pellets (hereinafter referred to as the chlorella diet) at Chlorella Kogyo Co., Ltd (Chikugo, Fukuoka, Japan). Lactose was used as the placebo. Since lactose has a lower volume per unit weight than chlorella and cannot be formulated into pellets identical in size to the chlorella diet, 240 mg of lactose (hereinafter referred to as the placebo diet) was used to produce pellets similar in shape and color to the chlorella diet (Table 1).

The chlorella and control groups consumed the chlorella and placebo diets, respectively, for 4 weeks. Subjects consumed 10 tablets 3 times a day after each meal for both chlorella (6 g) and placebo (7.2 g) diets. At the completion of the intervention period, we assessed the intake status of the subjects.

Fatigue exercise load

To simulate mild fatigue experienced in daily work, subjects underwent bicycle ergometer exercise with an intensity equivalent to 40% of the heart rate reserve (HRR) for 30 min (hereinafter referred to as fatigue exercise load) under the supervision of a physical therapist and a physician. The target heart rate during exercise was calculated based on the resting heart rate using the Karvonen formula (target heart rate = 0.4 [(220 - age) - resting heart rate] + resting heart rate).

Measurement parameters

1) F-VAS questionnaire: F-VAS questionnaire is recommended by the Japanese Society of Fatigue Science as a method of evaluating fatigue [15]. The self-assessment score is marked on a 10 cm line, with the left end (0 cm) representing “no fatigue at all” and the right end (10 cm) representing the “maximum level of fatigue experienced”. Subjects were requested to mark their level of fatigue on the scaled line.

2) Anthropometric measurements, pulse, and blood pressure: height was measured using a height gauge. Body weight and body mass index were measured using a dual frequency body composition monitor (DC-320, Tanita Corp., Tokyo, Japan). Blood pressure and heart rate were measured using the Omron automatic blood pressure HBP-9021 system (Kyoto, Japan), before and immediately after fatigue exercise load.

3) Blood examination: vanillylmandelic acid (VMA) and homovanillic acid (HVA) are the final metabolites of catecholamines and have been shown to be useful as biochemical indicators of fatigue-associated alterations of autonomic nervous function [16]. VMA and HVA measurements were performed using high performance liquid chromatography. The total antioxidant capacity was measured to evaluate the ability of antioxidants to remove active oxygen in the blood [17] using the OxiSelect total antioxidant capacity assay kit (Cell Biolabs Inc., San Diego, CA). Increased oxidative stress causes various forms of oxidative damage to proteins, including carbonyl modification resulting in carbonylated proteins [18]. The OxiSelect protein carbonyl enzyme-linked immunosorbent assay kit (Cell Biolabs Inc., San Diego, CA) was used to quantify protein carbonyls. Malondialdehyde (MDA) is a lipid oxidation degradation product and has been used as a marker of lipid peroxidation [19]. The MDA assay kit (JaICA, Shizuoka, Japan) was used to measure MDA.

Statistical analysis

Subjects were randomly assigned by the age-based

Table 1. Nutritional facts of Chlorella and Placebo (per 100 g)

| Composition name (Unit) | Chlorella | Placebo |
|------------------------|-----------|---------|
| Calorie (kcal)         | 390       | 392     |
| Protein (g)            | 62        | 0.8     |
| Lipids (g)             | 11        | 1.5     |
| Glucide (g)            | 1         | 93.8    |
| Dietary fiber (g)      | 11        | 0       |
| Chlorophyll (g)        | 3.2       | 0       |
| Sodium (mg)            | 15.4      | 0.2     |
| Iron (mg)              | 75        | 0.1     |
| Potassium (mg)         | 1000      | 1       |
| Magnesium (mg)         | 350       | 0.8     |
| Vitamin B1 (mg)        | 1.8       | 0       |
| Vitamin B2 (mg)        | 5         | 0       |
| Vitamin B6 (mg)        | 2         | 0       |
| Vitamin B12 (μg)       | 500       | 0       |
| Folic acid (μg)        | 2500      | 0       |
| Biotin (μg)            | 300       | 0       |
| Vitamin C (mg)         | 60        | 0       |
| Vitamin E (mg)         | 30        | 0       |
| Lutein (mg)            | 270       | 0       |
| α-carotene (mg)        | 7         | 0       |
| β-carotene (mg)        | 90        | 0       |
| Zeaxanthin (mg)        | 30        | 0       |
| Total weight (g)       | 100       | 100     |
permutated block method. However, due to the small sample size, the t-test and Wilcoxon test were used to compare subjects’ anthropometric measurements, blood pressure, heart rate, and blood test results before the intervention/fatigue exercise load. Effect indices, which were collected at four different time points, were analyzed using a linear mixed model considering the correlation between repeated measurements. Effects of measurements that showed a significant difference between groups at the baseline were adjusted in the model. SAS (version 9.4, SAS Institute Japan Ltd. Tokyo, Japan) was used for the linear mixed model, and SPSS (version 19, IBM Corp., Armonk, NY) was used for the remaining statistical analyses. All statistical analyses were one-tailed tests, and the significance level was set at <0.05.

RESULTS

Data on a total of 27 subjects, mean age 35.4±10.4 years, were analyzed. Baseline characteristics of the subjects are shown in Table 2. There were no significant differences between groups in any of the characteristics measured at baseline. No one reported performing excessive physical activity during the intervention period; all subjects complied with the test diet instructions, except that they sometimes took 10-20 tablets twice a day if they skipped a dose of the test diet.

F-VAS questionnaire

F-VAS measurements are shown in Table 3. At baseline, F-VAS significantly increased in both groups after fatigue exercise load. After the intervention period, however, F-VAS significantly increased after fatigue exercise load in the placebo group while no significant increase was observed in the chlorella group. There were no significant differences in F-VAS between the two groups.

Pulse and blood pressure

Pulse and blood pressure measurements are shown in Table 4. Pulse significantly increased after fatigue exercise load, and diastolic pressure decreased after test ingestion. There were no significant differences in systolic pressure between the two groups.

| Test (Unit) | Chlorella group | Placebo group | P value
|-------------|----------------|---------------|--------|
|            | Baseline       | Chlorella     |        |
| FatigueVAS (cm) |              |               |        |
| Rest        | 2.7±2.0        | 3.8±2.8       |        |
| Exercise    | 4.3±2.4*       | 4.9±2.0       | 4.9±1.7* |
|             | 5.1±2.3*       |               |        |

Mean±SD, *: Intragroup difference for exercise(p<0.05)

| Test (Unit) | Chlorella group | Placebo group | P value
|-------------|----------------|---------------|--------|
|            | Baseline       | Chlorella     |        |
| Pulse (bpm) |              |               |        |
| Rest        | 82.4±6.6       | 75.1±12.1     | 73.3±9.5 |
| Exercise    | 89.0±9.0*      | 88.6±9.6*     | 81.5±14.2 |
|             | 86.2±15.0*     |               |        |
| SBP (mmHg)  |              |               |        |
| Rest        | 133.9±14.3     | 132.0±18.7    | 134.0±13.8 |
| Exercise    | 134.9±20.8     | 128.3±15.3    | 127.2±11.6 |
|             | 134.8±12.3     |               |        |
| DBP (mmHg)  |              |               |        |
| Rest        | 85.9±8.4       | 79.6±9.1†     | 78.4±10.1 |
| Exercise    | 82.6±10.8      | 79.1±10.5     | 77.9±7.3 |
|             | 81.0±7.5       |               |        |

Mean ± SD, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, †: Intragroup difference for ingestion (p<0.05), *: Intragroup difference for exercise (p<0.05)
exercise load at baseline and after the intervention period, in both groups. At baseline, the systolic blood pressure was significantly decreased post-exercise compared to before exercise in the placebo group. After the intervention period, diastolic blood pressure before exercise significantly decreased from 85.9±8.4 mmHg to 79.6±9.1 mmHg in the chlorella group; however, no significant differences were observed in blood pressure after the placebo diet. There were no significant differences in pulse and blood pressure between the two groups.

Effect on VMA and HVA

The VMA level significantly increased after fatigue exercise load, at baseline, and after the intervention period in both groups (Table 5). The HVA level also significantly increased after fatigue exercise load in both groups, and at all blood sampling points, except at baseline in the chlorella group (Table 5). There were no significant differences in the VMA and HVA levels between the two groups.

**Effect on oxidative stress**

First, we compared each index under resting conditions (Figure 2). After intervention, the MDA levels increased in the placebo group (0.26±0.08 μmol/L), but were significantly lower in the chlorella group (0.2±0.09 μmol/L). The levels of carbonylated proteins did not significantly change before and after the intervention period in either group; furthermore, there were no significant differences in the levels of carbonylated proteins between the two groups. Antioxidant capacity significantly increased after the intervention period in the chlorella group (baseline, 1066±162

![Table 5](image)

| Test (Unit) | Chlorella group | Placebo group |
|------------|----------------|--------------|
| VMA (ng/mL) Rest | 7.1±2.2 | 6.8±1.3 | 9.1±3.3 | 10.0±3.4 |
| Exercise | 7.9±2.4* | 7.8±1.4* | 10.9±3.2* | 11.0±3.5* |
| HVA (ng/mL) Rest | 10.2±4.6 | 9.4±4.9 | 10.0±2.6 | 10.0±3.3 |
| Exercise | 10.7±3.8 | 11.1±5.9* | 11.4±3.2* | 11.5±3.5* |

Mean±SD, *: Intragroup difference for exercise (p<0.05), VMA: Vanillylmandelic acid, HVA: Homovanillic acid

![Figure 2](image)

Fig. 2. Comparison of oxidative stress. A: Malondialdehyde at the resting condition. * p<0.05 versus placebo after infection, ** p<0.05 versus Baseline; B: Carbonylated protein at the resting condition. No significant difference between group and intragroup; C: Total antioxidant capacity at the resting condition. * p<0.05 versus Baseline; D: Malondialdehyde under the fatigue condition. No significant difference between group and intragroup; E: Carbonylated protein under the fatigue condition. * p<0.05 versus Baseline; F: Total antioxidant capacity under the fatigue condition. No significant difference between group and intragroup.
μmol/L; and after the chlorella diet, 1119±180 μmol/L), but not in the placebo group.

Next, we compared each index after fatigue exercise load (at fatigue condition; Figure 2). The levels of carbonylated proteins significantly increased after the intervention period in the placebo group, but not in the chlorella group. The MDA levels and antioxidant capacity did not significantly change before and after the intervention period in either group.

There were no significant differences in any of the oxidative-stress indices measured pre- and post-exercise either before or after intervention in either group.

**DISCUSSION**

Our study indicates that under resting conditions, the chlorella diet significantly increased the serum antioxidant capacity and decreased serum MDA levels. Although MDA levels at baseline were similar in both groups, MDA levels after the intervention period were significantly lower in the chlorella group than in the placebo group. The increased antioxidant capacity indicates an increase in antioxidant levels, which in turn inhibit oxidation of body tissues, including lipids, while the decreased MDA level indicates a reduction in lipid oxidation. It has been reported that chlorella is rich in lutein, an antioxidant, and the lutein concentration in blood can be effectively maintained by regular oral ingestion of chlorella [12]. Several previous human studies show that similar to chlorella, the ingestion of watercress containing substantial amounts of carotenoids, such as lutein, attenuates exercise-induced DNA-damage and lipid peroxidation in peripheral mononuclear cells [20]. Furthermore, in a murine model of age-dependent dementia, a chlorella diet tended to reduce oxidative stress and had a significant preventative effect on cognitive decline [13]; likewise, protective effects of chlorella against lead-induced oxidative stress have been observed in the brains of rats [14]. Our findings are consistent with these previous studies and suggest that a chlorella diet may exert favorable effects on the redox balance as a source of antioxidants.

Post exercise, under fatigue condition, the placebo group showed a significant increase in levels of carbonylated proteins after the intervention period; however, no significant changes in the levels of carbonylated proteins were observed in the chlorella group. One previous human study determined that carbonylated proteins levels increased after one bout of exhaustive exercise load regardless of exercise experience, and this increase was diminished by the ingestion of antioxidant vitamins [21]. These results support our findings suggesting that chlorella may inhibit exercise-induced protein oxidation in a manner similar to that of antioxidant vitamins. However, we could not show clear effects of chlorella on oxidative stress under fatigue condition since no significant changes were observed in oxidative stress indices after exercise in either group.

In this study, to simulate fatigue caused by daily physical workload, subjects underwent fatigue exercise loading. To simulate mild fatigue experienced in daily work, we adjusted the duration and intensity of the exercise such that subjects would not experience excessive fatigue loading. F-VAS significantly increased in both groups under fatigue condition, compared to the resting condition, except after the chlorella diet. Further, a small but significant increase occurred in the post-exercise pulse, VMA, and HVA levels compared to the resting condition at all measurements in both groups. These observations suggest that we were able to successfully reproduce a mild fatigue state using the fatigue exercise load in the present study. However, no significant changes in oxidative stress indices were observed after the fatigue exercise load compared to the resting condition in either group. In the present study, one bout of low intensity physical exercise did not induce significant oxidative stress, probably because the level of RONS did not overwhelm defense mechanisms. Alternatively, it is possible that RONS produced during exercise may have occurred within tissues (e.g., skeletal muscle, cardiac, liver, and brain) other than the blood. Since we did not measure the cellular levels of oxidative-stress markers, low levels of RONS following a low intensity exercise (40% HRR) might not have been detected; although, under the same condition, increased F-VAS, VMA, and HVA were detected.

The measurements under fatigue conditions are comparable to the evaluation of acute fatigue, however we were unable to clarify the effect of chlorella on oxidative stress under acute fatigue conditions. Although the subjects in this study did not experience chronic fatigue, the results of this study showed that chlorella ingestion improved oxidative stress and fatigue symptoms under resting conditions; hence, chlorella may have favorable effects on chronic fatigue.

We did not detect any significant changes in the levels of VMA and HVA before and after chlorella ingestion. VMA and HVA are biochemical indicators of fatigue-associated alterations of autonomic nervous function; thus, chlorella does not seem to affect the autonomic nervous function. In contrast, F-VAS, which
is a subjective marker of fatigue, was not significantly increased after exercise in the chlorella group, although F-VAS increased significantly post-exercise at baseline in both groups, and after the intervention period in the placebo group. A previous study also showed that the chlorella diet reduced fatigue symptoms in patients with breast cancer [22]. Although the cause of fatigue was different, those results are consistent with our findings suggesting that chlorella ingestion may enhance tolerance for fatigue.

In the chlorella group, there was a small but significant decrease in the diastolic blood pressure in the resting condition after chlorella ingestion compared to before ingestion. The function of the renin-angiotensin system is well known in blood pressure regulation. Chlorella has been reported to inhibit the angiotensin I-converting enzyme [23]. Moreover, chlorella has been reported to decrease blood pressure in hypertensive rats [24]. However, in the current study, we did not detect any significant changes in systolic blood pressure before and after chlorella ingestion; thus, chlorella does not seem to have affected the blood pressure.

Limitations

We excluded women from the study to eliminate the effects of the estradiol cycle on fatigue symptoms. However, since physical and mental fatigue in daily life are experienced by both men and women, it is necessary to extend the range of the study to women. Furthermore, because of the small sample size, we could not show clear differences between the placebo and chlorella groups even though there were significant intragroup differences before and after the intervention period in several parameters. Therefore, a large-scale investigation including women is warranted to clearly elucidate the effect of chlorella ingestion on oxidative stress and consequent fatigue. Because the subjects were volunteers, most subjects might have been health conscious.

The extent of physical activity during the research period was self-reported. In order to standardize physical activity of subjects, detailed evaluation was necessary. Undeclared changes in working hours or work intensity might be a cause of concern in the evaluation of subjective fatigue.

Conclusions

Chlorella ingestion increased the antioxidant capacity and decreased reactive-oxygen metabolites under resting conditions, suggesting that it has a potential to relieve oxidative stress and enhance tolerance for fatigue.

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