Original Article

Chlorella-derived multicomponent supplementation increases aerobic endurance capacity in young individuals

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Chlorella, a unicellular green alga, contains a variety of nutrients including amino acids, carbohydrates, vitamins, and minerals. A previous animal study found that maximal swimming time in mice increased after 14 days on a diet including _Chlorella_ powder compared to no change in swimming performance on a normal diet. However, it is currently unknown whether _Chlorella_-derived multicomponent supplementation increases aerobic endurance capacity in humans. We investigated the effects of _Chlorella_-derived supplementation on peak oxygen uptake during incremental maximal cycling in young individuals using a double-blinded, placebo-controlled, crossover study design. Seven men and three women (mean age, 21.3 year) were allocated to placebo or _Chlorella_ tablets (15 tablets × twice per day) for 4 weeks, with at least a 6-week washout period between trials, in a randomized order. Peak oxygen uptake significantly increased after _Chlorella_ supplementation (before vs after, 37.9 ± 1.9 vs 41.4 ± 1.9 ml/kg/min, $p = 0.003$), but not with placebo (39.4 ± 2.2 vs 40.1 ± 2.1 ml/kg/min, $p = 0.38$). The change in peak oxygen uptake over the 4-week trial was significantly greater in the _Chlorella_ trial than in the placebo trial (3.5 ± 0.9 vs 0.7 ± 0.8 ml/kg/min, $p = 0.03$). These results suggest that _Chlorella_-derived multicomponent supplementation increases aerobic endurance capacity in young individuals.

Key Words: **alga, _Chlorella_, multicomponent supplementation, peak oxygen uptake**

Some multicomponent supplements have been reported to increase aerobic endurance capacity. Vaz *et al.*[1] reported that a supplement containing multivitamins and minerals improves peak oxygen uptake ($\dot{V}O_2$peak) in a group of nutritionally compromised children with a prevalence of red blood cell riboflavin deficiency of 87.7% and niacin deficiency of 75.6%. Knechtle *et al.*[2] demonstrated that performance in a multistage ultra-endurance run (17 days, 1,205 km) was greater in athletes using a multimineral supplement than those without the supplement, although this effect did not reach statistical significance ($p = 0.06$). However, these previous studies investigated idiosyncratic populations or exercises. When daily intake exceeds the recommended dietary allowance (RDA), Weight *et al.*[3,4] have reported that supplementation with vitamins and minerals do not improve blood levels of supplemented nutrients or aerobic endurance capacity. A more effective supplement may be needed to improve aerobic endurance capacity in individuals without overt nutrition deficiencies or involved in more common exercise regimens.

Broadening the ingredients in multicomponent supplements may improve aerobic exercise performance not only in individuals with nutrition deficiencies or during extreme exercises but also in individuals with no overt malnutrition or while performing more common exercises. For example, a previous animal study reported that maximal swimming time in mice increased after 14 days on a diet including *Chlorella* powder compared to no change in swimming performance on a normal (i.e., presumably non-deficient) diet.[5] *Chlorella* is a unicellular green alga that grows in fresh water and contains a variety of nutrients including amino acids (e.g., branched-chain amino acids [BCAAs] and arginine), carbohydrates, vitamins, and minerals.[6,7] BCAAs and arginine can potentially increase aerobic endurance performance in individuals without overt nutritional deficiencies.[8–12] Carbohydrates were found to be an effective supplement for enhancing aerobic endurance performance in a comprehensive review by the International Society of Sports Nutrition (ISSN).[13] However, it remains unexplored whether *Chlorella*-derived multicomponent supplementation increases aerobic endurance capacity in humans. We investigated the effects of *Chlorella*-derived supplement on $\dot{V}O_2$peak during a graded maximal cycling test in young individuals using a double-blinded, placebo-controlled, randomized, crossover study design.

Materials and Methods

Participants. The participants who volunteered to be in this study included young men ($n = 7$) and women ($n = 3$). Nine subjects did not use any dietary supplements on a regular basis; one man was taking a protein supplement two to three times a week. Six subjects engaged in exercise regularly (more than 30 min/day on 3 days/week). None of the subjects were smokers. The participants were asked not to modify their lifestyle during each trial period. The mean ± SE values for age and height were 21.3 ± 0.3 years and 1.67 ± 0.02 months, respectively.

This study was approved by the Ethical Committee of Ryutsu Keizai University and conformed to the principles outlined in the Helsinki Declaration. All participants gave their written informed consent before participation.

Experimental design. Each participant took part in the placebo and *Chlorella* trials in a randomized order. First, a maximal exercise test was performed. The following day, participants were randomized into either the placebo or *Chlorella* (Sun Chlorella A; Sun Chlorella Corp., Kyoto, Japan) trial in a double-blinded manner. During each trial, subjects took 30 tablets per day (15 tablets twice daily, after breakfast and dinner) for 4 weeks.[6,7,14] This dosage is in accordance with the general recommended dosage for Japanese consumers. Exercise testing was done 1 day after the final tablet intake. After a washout period of at least 6 weeks, the second trial commenced with the alternate trial assignment.

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Table 1. Body weight, body mass index, and peak values of heart rate, respiratory exchange ratio, rating of perceived exertion, and work rate during exercise testing before and after *Chlorella* or placebo intake

		Before	After	Interaction
Body weight (kg)	Placebo	65.0 ± 3.2	64.5 ± 2.8	*F* = 0.2
	Chlorella	64.9 ± 2.9	64.6 ± 2.7	*p* = 0.65
Body mass index (kg/m²)	Placebo	23.4 ± 1.2	23.2 ± 1.1	*F* = 0.3
	Chlorella	23.3 ± 1.0	23.3 ± 0.9	*p* = 0.60
Peak heart rate (bpm)	Placebo	183 ± 4	183 ± 3	*F* = 0.0
	Chlorella	186 ± 4	186 ± 3	*p* = 0.97
Peak respiratory exchange ratio	Placebo	1.25 ± 0.02	1.25 ± 0.02	*F* = 0.0
	Chlorella	1.24 ± 0.02	1.24 ± 0.02	*p* = 0.82
Peak rating of perceived exertion (Borg's 6–20 scale)	Placebo	17.9 ± 0.6	18.5 ± 0.5	*F* = 0.0
	Chlorella	18.2 ± 0.6	18.7 ± 0.5	*p* = 0.89
Peak work rate (W)	Placebo	220 ± 15	224 ± 13	*F* = 1.7
	Chlorella	211 ± 14	225 ± 15	*p* = 0.20

Values are means ± SE.

Placebo and *Chlorella* tablet. The placebo and *Chlorella* tablets used in this study were the same as in our previous studies.[6,7,14] The mass of each tablet was 243 and 200 mg, respectively. The main components of the placebo tablet were lactose and colorant. The main ingredient of the *Chlorella* tablet was dried *Chlorella pyrenoidosa* powder. The nutritional value per day of the placebo and *Chlorella* supplementations were, respectively: energy, 29.6 and 23.9 kcal; water, 0.2 and 0.3 g; protein, 0.1 and 3.6 g; lipid, 0.4 and 0.6 g; sugar, 6.2 and 0.4 g; dietary fiber, 80 and 714 mg; and ash, 160 and 390 mg. The color and shape of the placebo and *Chlorella* tablets were similar.

Maximal exercise testing. Maximal exercise testing consisted of incremental cycling to exhaustion (4 min at 70 W [men] or 50 W [women], with a 30 W [men] or 20 W [women] increase every 2 min) with monitoring of breath-by-breath oxygen uptake and carbon dioxide production (AE300S; Minato Medical Science, Osaka, Japan), heart rate (HR) calculated by 3-lead electrocardiography (LRR-03; GMS, Tokyo, Japan), and ratings of perceived exertion (RPE, Borg's 6–20 scale). We considered a subject as having achieved maximal exertion when at least 2 of the following 4 criteria were met: 1) a plateau in oxygen uptake with increasing exercise intensity (<100 ml/min); 2) achievement of age-predicted maximal HR (±10 bpm); 3) a respiratory exchange ratio (RER) of at least 1.15; and 4) a RPE of at least 18 units.

Statistical analysis. Results are given as means ± SE. *p* values<0.05 were considered statistically significant. Inter-trial differences in values before each intake period were analyzed using *t* tests. To compare the effects of *Chlorella* intake with placebo supplementation, repeated-measures analysis of variance (ANOVA) was used. Changes in parameters during the 4-week intake period were compared between the trials using analysis of covariance (ANCOVA), which included the pre-intake value as a covariate. When a significant *F* value in ANOVA or ANCOVA was observed, a post hoc Fisher's test was used to identify the effects of placebo and *Chlorella* intake.

Results

Before tablet intake, there were no significant differences between the placebo and *Chlorella* trials in all parameters (t = 0.0–0.5, p = 0.61–0.98), including $\dot{V}O_2$peak (39.4 ± 2.2 vs 37.9 ± 1.9 ml/kg/min, t = 0.5, p = 0.61). Additionally, there were no significant differences between the first and second trials in all pre-intake variables (t = 0.0–1.4, p = 0.16–0.96).

There were no subjects who were discriminating the placebo tablet from the *Chlorella* tablet, although there was a difference in taste. There were no significant interactions between trial and supplementation period in the parameters of Table 1. A significant interaction (trial × intake period) was identified in $\dot{V}O_2$peak (F = 5.4, p = 0.03, Fig. 1, left panel). Compared to baseline, $\dot{V}O_2$peak was significantly higher after *Chlorella* intake (41.4 ± 1.9 ml/kg/min) whereas there was no significant change in $\dot{V}O_2$peak with placebo (after intake; 40.1 ± 2.1 ml/kg/min, F = 0.8, p = 0.38). The change in $\dot{V}O_2$peak over the 4-week trial was significantly greater in the *Chlorella* trial than in the placebo trial (3.5 ± 0.9 vs 0.7 ± 0.8 ml/kg/min, Fig. 1, right panel).

All subjects in both trials satisfied the criteria for maximal exertion during pre-intake exercise testing, but one subject in each trial could not achieve maximal exertion during post-intake testing. Results with these two subjects excluded (n = 8) were nearly identical to results with all subjects included. A significant interaction between trial and intake period was identified in maximal oxygen uptake ($\dot{V}O_2$max; F = 10.8, p = 0.005). Additionally, a significant interaction was identified in maximal work rate (WRmax; F = 5.1, p = 0.04). $\dot{V}O_2$max and WRmax significantly increased after *Chlorella* intake (before, 37.3 ± 2.2 vs after, 41.6 ± 2.3 ml/kg/min, F = 23.3, p = 0.001 and 216 ± 15 vs 234 ± 16 W, F = 11.1, p = 0.01; respectively), but there were no significant differences in these parameters with placebo (40.7 ± 2.6 vs 40.9 ± 2.6 ml/kg/min, F = 0.1, p = 0.81 and 230 ± 16 vs 230 ± 16 W, F = 0.0, p>0.99; respectively). The change in $\dot{V}O_2$max during the 4-week trial was significantly greater in the *Chlorella* trial than in the placebo trial (4.3 ± 0.9 vs 0.2 ± 0.9 ml/kg/min, F = 8.7, p = 0.006). Inter-trial differences in the change in WRmax did not reach statistical significance (18 ± 5 vs 0 ± 6 W, F = 4.4, p = 0.05).

Discussion

This is the first study investigating the effects of *Chlorella*-derived multicomponent supplement on aerobic endurance capacity in humans. We demonstrated that $\dot{V}O_2$peak during graded maximal exercise testing increased after 4 weeks of *Chlorella* supplementation, but not with placebo. Results with absolute $\dot{V}O_2$peak are not shown, but were same as the present results (i.e., the results with $\dot{V}O_2$peak per body weight). These results suggest that supplementation with a *Chlorella*-derived multicomponent supplement increases aerobic endurance capacity in young individuals.

Although we could not investigate mechanisms underlying *Chlorella* tablet-induced change in $\dot{V}O_2$peak, we have hypothesized several mechanisms. The difference in the dose between the placebo and *Chlorella* trials was more marked in protein than other nutrients. Some amino acids directly or indirectly participate

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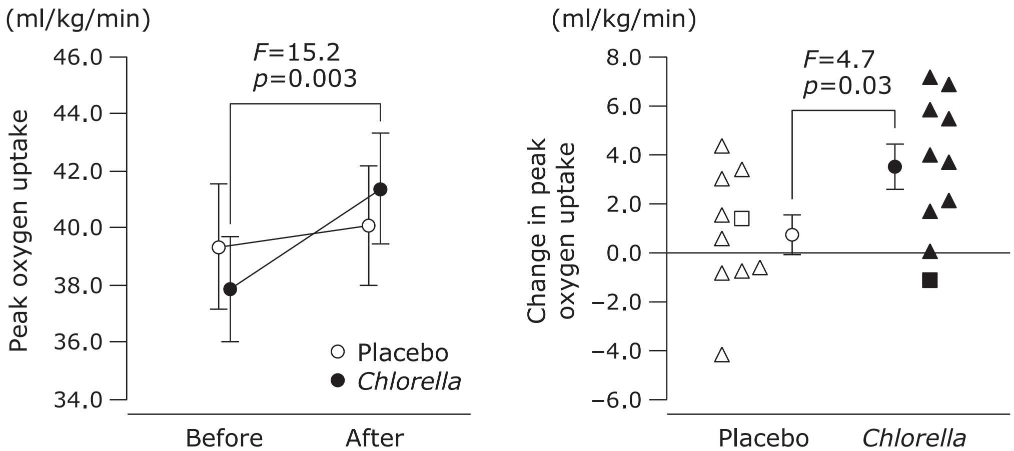

Fig. 1. Peak oxygen uptake before and after *Chlorella* or placebo intake (left panel) and change in peak oxygen uptake with *Chlorella* or placebo supplementation (right panel). Circles, means ± SE; triangles, subjects who achieved maximal exertion during both pre- and post-intake exercise testing; squares, subjects who achieved maximal exertion during pre-intake testing but not during post-intake testing.

in energy metabolism and can affect aerobic endurance capacity. BCAAs have been categorized as possibly effective supplements in the ISSN review, based on the rationale that ingestion of BCAAs during prolonged exercise could theoretically ameliorate central fatigue.[13] Indeed, supplementation with BCAAs (300 mg/kg/day, corresponding to 19,500 mg/day for our subjects or 29.4 mg/kg during pre-exercise resting period and every 30 min during exercise, corresponding to 13,500 mg/day for our subjects) or combined intake of BCAAs and arginine (2,000 mg/day and 500 mg/day, respectively) have been reported to increase aerobic endurance performance during sub-maximal and maximal exercise,[10–12] although there have been some conflicting reports.[13] Arginine is converted to nitric oxide,[15,16] an endothelium-derived vasodilator, which can increase blood flow to muscles. Some previous studies have demonstrated that supplementation with arginine (3,000 or 6,000 mg/day) improves oxygen cost, aerobic capacity, and time to exhaustion during submaximal exercise,[8,9] although one study reported that arginine supplementation (15,000 mg/day) did not change endurance capacity.[17] A dose of ash was also greater in the *Chlorella* tablet than the placebo tablet. A previous study demonstrated that 8 weeks of vitamins B1, B2, B6, and C restriction (21.3–32.5% of RDA) lowers $\dot{V}O_2max$ in healthy young men, but supplementation with these nutrients (twice the RDA; 2,500 µg/day, 4,000 µg/day, 4,000 µg/day, and 100,000 µg/day, respectively) prevents the reduction of $\dot{V}O_2max$ induced by the restrictive diet.[18] It has been well established that supplementation with vitamin C and iron can improve iron-deficiency anemia; the general dose of iron is 100–200 mg/day. Multicomponent analysis of the *Chlorella* tablet showed that it contains BCAAs (571 mg/30 tablets [i.e., mg/day]), arginine (190 mg/30 tablets), vitamins B6 (126 µg/30 tablets) and C (240 µg/30 tablets), and iron (8.4 mg/30 tablets).[6,7] In addition, we have observed that *Chlorella* tablets contain vitamins B1 and B2 (112 and 337 µg/30 tablets, respectively; unpublished data). It is possible that one or some of these nutrients are associated with increase in $\dot{V}O_2peak$ after *Chlorella* supplementation. However, the dose of each nutrient in the *Chlorella* tablets was markedly smaller than the dose in the previous studies. On the other hand, we have found more than 50 nutrients in the previous multicomponent analyses of the *Chlorella* tablet (unpublished data). Improving aerobic endurance capacity with *Chlorella* supplementation might be due to a wide spectrum of nutrients rather than bioactive effects of some particular nutrients.

The previous study, which found 13,683 genes expression after forced swimming in mice, reported that 774 genes were downregulated and 220 genes were upregulated in *Chlorella* intake mice, whereas the 774 genes were upregulated and the 220 genes were downregulated in normal diet mice.[5] Immune response- and oxidoreductase activity-associated genes were included in the downregulated genes with *Chlorella* supplementation and angiogenesis-related genes were included in the upregulated genes. Although it is currently unknown which genes were implicated in an increase in maximal swimming time in mice on a diet including *Chlorella* powder, these genes may provide keys to elucidate the mechanisms underlying an improvement of aerobic endurance capacity with *Chlorella* supplementation.

Since mice in the control group were fed a standard diet, it is reasonable to hypothesize that there were no malnourished mice in the previous study investigating effects of *Chlorella* supplementation on maximal swimming time.[5] However, it is possible that subjects with malnutrition were included in our human study. Many studies have reported that skipping breakfast, eating fast food, and dieting to lose weight may lead to malnutrition in young adults.[19–24] In particular, university students living away from home are at a higher risk for poor nutrition.[25] The subjects in this study were undergraduates and graduate students; 8 of them were living away from home. The participants might have inadequate nutritional status, and replenishment of deficient nutrients with *Chlorella*-derived supplementation might have elicited an improvement in aerobic endurance capacity. However, it is a speculation to assume that there were subjects with nutrition deficiencies in this study. Additionally, it is unclear that the *Chlorella*-derived tablet has sufficient dose to restore the deficiencies. Further studies are warranted to elucidate associations of pre-intake nutritional status with effects of *Chlorella* supplementation.

There were two subjects who did not achieve maximal exertion during the post-intake exercise test, one in each trial. The subject in the placebo trial met one criterion (RPE≥18) and nearly met a second criterion (peak RER, 1.14). The effect of this subject's exertion level on the mean values in the placebo trial might be insignificant. On the other hand, the subject in the *Chlorella* trial did not meet any of the maximal exertion criteria. If this subject had also achieved maximal exertion after *Chlorella* intake, the effects of *Chlorella* intake on aerobic endurance capacity may be clearer. Indeed, the *p* values were lower in the analyses that excluded these two subjects ($\dot{V}O_2max$) than the analyses that included these subjects ($\dot{V}O_2peak$).

We used lactose as a main component (i.e., 80%) of the placebo tablet.[14] Consequently, the placebo tablet contained much more of sugar than the *Chlorella* tablet. Carbohydrates are one of the best ergogenic aids; athletes should intake more carbohydrates to maintain liver and muscle glycogen stores.[13] This ISSN review says that active individuals should consume carbohydrates at a dose of 5–8 g/kg/day; the active subjects in the present study are recommended to consume carbohydrates of 325–520 g/day. Although the dose of carbohydrates in the placebo trial was slight (<2.0% of the recommendation) and the placebo intake did not increase $\dot{V}O_2$peak, we should note the possibility that sugar in the placebo tablet affected aerobic endurance capacity. The effects of *Chlorella* intake on aerobic exercise performance might be clearer if the placebo tablet contained less sugar.

This study has the following limitations. First, we could not collect information regarding dietary habit and nutritional status. This information is needed to discuss mechanisms underlying *Chlorella* tablet-induced changes in aerobic endurance capacity. Second, we cannot rule out the possibility that exercise habit changed during the study period, although the participants were asked not to modify their lifestyle during each trial period and there were no differences in pre-intake parameters between the first and second trials. Finally, the sample size in this study was small and not equal between men and women. Additionally, exercise training history of the subjects was heterogeneous. The present results should be confirmed with larger study populations. Effects of *Chlorella* supplementation in each sex, in each exercise training history, and in each physical fitness level have also remained to be investigated.

In summary, the novel finding of this double-blinded, placebo-controlled, randomized, crossover study of young individuals was that *Chlorella*-derived multicomponent supplementation for 4 weeks increases $\dot{V}O_2$peak during incremental maximal cycling. This result suggests that *Chlorella*-derived multicomponent supplementation increases aerobic endurance capacity in young individuals.

Abbreviations

ANCOVA	analysis of covariance
ANOVA	analysis of variance
BCAAs	branched-chain amino acids
HR	heart rate
ISSN	The International Society of Sports Nutrition
RDA	recommended dietary allowance
RER	respiratory exchange ratio
RPE	ratings of perceived exertion
$\dot{V}O_2$max	maximal oxygen uptake
$\dot{V}O_2$peak	peak oxygen uptake
WRmax	maximal work rate

Conflicts of Interest

SunChlorella Co., Ltd. provided funding and the test supplements. T.O. received a speaker's honorarium from SunChlorella Co., Ltd. S.U. declares no conflict of interest.

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