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ORIGINAL ARTICLE

Preventive effects of *Spirulina platensis* on skeletal muscle damage under exercise-induced oxidative stress

Hsueh-Kuan Lu · Chin-Cheng Hsieh · Jen-Jung Hsu · Yuh-Kuan Yang · Hong-Nong Chou

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Abstract The effects of spirulina supplementation on preventing skeletal muscle damage on untrained human beings were examined. Sixteen students volunteered to take *Spirulina platensis* in addition to their normal diet for 3-weeks. Blood samples were taken after finishing the Bruce incremental treadmill exercise before and after treatment. The results showed that plasma concentrations of malondialdehyde (MDA) were significantly decreased after supplementation with spirulina ($P < 0.05$). The activity of blood superoxide dismutase (SOD) was significantly raised after supplementation with spirulina or soy protein ($P < 0.05$). Both of the blood glutathione peroxidaes (GP_x) and lactate dehydrogenase (LDH) levels were significantly different between spirulina and soy protein supplementation by an ANCOVA analysis ($P < 0.05$). In addition, the lactate (LA) concentration was higher and the time to exhaustion (TE) was significantly extended in the spirulina trail ($P < 0.05$). These results suggest that ingestion of *S. platensis* showed preventive effect of the skeletal muscle damage and that probably led to postponement of the time of exhaustion during the all-out exercise.

Keywords Algae · Antioxidant · Lactate dehydrogenase · Fatigue · Malondialdehyde

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Introduction

We are interested in the reason why in Nagpur, India athletes at an orphanage have been eating spirulina, a blue–green alga or cyanobacterium, regularly while training for track and field events (Fox 1996). The Chinese and the Cuban Olympic teams eat spirulina daily during their training and before competition (Huang et al. 2000). It is probably due to the aspect of having the effect on promoting health and/or on exercise performance by using spirulina as dietary supplement. However, there is still no evidence on the exercise-related advantages of supplementing spirulina in humans.

Spirulina platensis, along with its related species (*Spirulina maxima*), is well known as a protein-rich food for dietary supplement (Vonshak 1997). This species and many other mass-cultivated or harvested species for food (Fox 1996), feed (Belay et al. 1996), or fine chemical (Tanticharoen et al. 1994) sources, are actually classified into the Genus *Arthrospira*. However, the term spirulina, even representing another taxonomic group, is still being used to name these organisms, traditionally mass-cultivated and sold in the market. Due to the lack of cellulose cell wall, spirulina has a better digestibility than *Chlorella*, another well-known group of algal species. Except the character of high-nutritive quality in protein content, essential amino acid composition, gamma-linolenic acid content, and branch chain amino acid (BCAA) content,

spirulina is nontoxic to the tested animals or human beings (Fox 1996). Such beneficial characteristics and special handling in culture maintenance have made spirulina an emerging product in algal industry following *Chlorella*.

In addition to the nutritional value of spirulina, many reports have claimed its bioactive functions arising from the contents of this organism. Prevention of fatty liver (Rodriguez-Hernandez et al. 2001), cardiovascular disease (Paredes-Carbajal et al. 2001), cancers (Mathew et al. 1995), reducing serum lipid level in serum (Mani et al. 2000), elevating blood hemoglobin level (Uliyar et al. 2000), antibody production (Hayashi et al. 1998) and macrophage phagocytic function (Al-Batshan et al. 2001), inhibiting HIV virus replication (Hayashi et al. 1996), and anti-oxidation (Farooq et al. 2004) were, respectively, published. Spirulina could benefit health by reducing exercise-induced muscle damages in rats (Huang et al. 2000). Polysaccharide from spirulina is a main anti-fatigue material during endurance exercise in mice (Zhang and Liu 1999).

Many products such as carnitine (Kanter and Williamhe 1995), vitamin C (Goldfarb 1995), vitamin E (Goldfarb 1995; Beaton et al. 2002), co-enzyme Q10 (Svensson et al. 1999), zinc (Micheletti et al. 2001), colecithin (Wu et al. 2000), proanthocyanidin (Bagchi et al. 2000), creatine (Powers et al. 2004), polyphenols (Matuschek and Svanberg 2002), astaxanthin (Chien et al. 2003), and beta-carotine (Gireesh et al. 2004), etc. were shown to have significant effect on anti-oxidation. Only creatine has reported the effects both on anti-oxidation and exercise performance (Powers et al. 2004). Creatine in the form of phosphocreatine is an important store of energy in muscle cells. During intense exercise, phosphocreatine is broken down to creatine and phosphate, and the energy released is used to regenerate the primary source of energy, adenosine triphosphate (ATP). Except having the characterization of anti-oxidation being the same as creatine, the nutritional value with many other bioactive functions on spirulina as mentioned above engage our interest much both on the research of exercise health and exercise performance. In this study, the effects of spirulina supplementation on preventing skeletal muscle damage on untrained humans were examined. Moreover, a passing mention is made on delaying fatigue by taking spirulina and is discussed.

Methods

Participants

Sixteen college students volunteered in this study. Subjects were informed of the risks and benefits of participation before signing the consent. This study was approved by the ethical committee of National Taiwan College of Physical Education who further approved the protocols therein. Prior to the experiment, all subjects were asked to record dietary intake for 4 days including 1 weekend day; Weighed food and calculated according to 'Taiwan Ordinary Food Nutrition Atlas'. The physical characteristics of subjects and their daily nutritional analysis are shown in Table 1. All other data collected in this study were based on double-blind experiments. Sixteen subjects were randomly divided into two groups. There were three men and five women in each group. The experimental group was given commercial pure product *Spirulina* Fareast Microalgae Industry (FEMICO) as dietary supplement. The control group was given an equal amount of soy protein (protein 53.3 and carbohydrate 33.3%).

Either *Spirulina* or soy protein was given in capsules (0.5 g each). Each subject was asked to take five capsules before each meal, three meals daily. The daily dosage was 7.5 g, below the normally recommended 10 g (Fox 1996). Three weeks of the supplementation period were determined previously by our pilot studies. Blood of these volunteers was drawn for analysis on the day before taking the diet supplement and the last day of treatment. Subjects performed the all-out treadmill exercise following the Bruce incremental protocol (Bruce 1972). Thirty minutes later, blood samples were taken via venous puncture. The respiratory valuables were monitored with a Vmax-29C (Sensormedics, CA, USA) gas analyzer. The exhaustive extent on a treadmill was dependent both by the rating of perceived exertion and the value of respiratory quotient (RQ).

Blood treatment and analysis

Eight milliliter venous blood samples were drawn at the time of 30 min after exhaustive treadmill exercise.

Table 1 Physical characteristics of the tested individuals

Characteristic	Spirulina	Placebo
Age (year)	20.00 ± 0.69	21.43 ± 1.02
Weight (kg)	60.00 ± 4.64	55.60 ± 2.23
Height (m)	1.63 ± 0.02	1.63 ± 0.02
BMI (kg/m^2)	22.67 ± 1.77	20.87 ± 1.34
Nutritional analysis (habitual daily intake)		
Energy (kcal)	2256.97 ± 52.41	2235.16 ± 30.43
Protein (g)	82.42 ± 2.45	87.20 ± 4.04
Fat (g)	66.30 ± 2.33	72.66 ± 2.77
CHO (g)	324.54 ± 14.46	304.02 ± 10.23

Values for each group represent mean ± SE (n = 8)

Blood samples were collected using vacuumed anticoagulant EDTA (0.1 mmol/l) tube, and then centrifuged at 3,000 rpm for plasma collection. Plasma samples were frozen at –70°C before analysis. Malondialdehyde (MDA) was measured by using Jain's method (Jain 1988). Total creatine kinase (CK-NAC, Randox kit numbers: CK110, CK 335, CK 522, CK 112, and CK 113), lactate dehydrogenase (LDH, Randox kit numbers: LD 357, LD347, and LD345), lactate (LA, Randox kit numbers: LD 1655, LD1618, and LD1684), superoxide dismutase (SOD, Randox kit numbers: SD124 and SD 125), and glutathione peroxidaes (GPx, Randox kit numbers: RS 504, RS 505, RS 506, and MS 181) were analyzed according to the standard spectrophotometric–colorimetric procedures provided with the commercial kits using a Shimadzu CL-770 (Shimadzu, Kyoto, Japan) spectrophotometer. The urine pH was assayed by the Miditron Junior II (Roche, Germany) analyzer.

Statistics

Statistical analyses were performed on SPSS (version 12.0). Results are expressed as mean ± standard error (SE). An unpaired Student's *t* with one-tail test was used to compare between groups before tests. A paired Student's *t* with one-tail *t*-test was used for the statistical analysis to compare the mean difference between pre- and post-tests within group. The sets of data in which there was significance between time and supplementation interaction were tested by the ANCOVA test. Probability levels (P) of significance of $P < 0.05$ and $P < 0.01$ for the type I error was considered significant and highly significant, respectively.

Results

General physical data and the average daily nutrient intake of subjects in the test are listed in Table 1. All of the others valuables including CK, LDH, SOD, GPx, MDA, LA, pH of urine, and the time to exhausted (TE) before diet supplementation between groups showed no significant difference. Each individual of LA (range from 5.01 to 24.71 mg/dl in the spirulina group and from 17.37 to 47.05 mg/dl in the placebo group) and GP_x (range from 13.72 to 58.83 u/gHb in the spirulina group and from 10.56 to 47.10 u/gHb in the placebo group) within the group showed large deviation in their figures leaded to the average of these two groups showed with no significant difference before diet supplement.

Table 2 showed the changes in basal parameters of SOD, GP_x, MDA, CK, LDH, LA, pH of Urine, RQ, and TE in spirulina- and placebo-supplemented subjects before and after 3-week nutritional intervention. The paired Student's *t*-test showed significantly increased effects on SOD parameters between pre- and post-tests within groups of both spirulina- and placebo-supplementation. The *spirulina* group significantly decreased MDA levels and significantly increased LA and TE levels after 3-week nutritional intervention. A significant interaction between time and supplementation was observed in GP_x and LDH levels by the ANCOVA test.

Table 2 Change in basal parameters of superoxide dismutase (SOD), glutathione peroxidaes (GP_x), malondialdehyde (MDA), creatine kinase (CK), lactate dehydrogenase (LDH), lactic acid (LA), pH of urine, respiratory quotient (RQ), and time to exhaustion (TE) in spirulina- and placebo-supplemented subjects before and after 3-week nutritional intervention

Variable	Before	After	Sb × Sa Pb × Pa	T × S
MDA (nmol/ml)				
Spirulina	56.21 ± 3.36	50.37 ± 4.35	0.033*	ns
Placebo	54.31 ± 2.69	55.08 ± 3.31	ns	
SOD (u/gHb)				
Spirulina	1324.09 ± 131.13	1852.45 ± 203.98	0.008**	ns
Placebo	1251.10 ± 77.26	1510.10 ± 48.94	0.018*	
GP_x (u/gHb)				
Spirulina	29.01 ± 5.18	33.86 ± 3.27	ns	0.018*
Placebo	21.32 ± 4.03	22.15 ± 2.07	ns	
CK (u/l)				
Spirulina	71.82 ± 17.58	51.16 ± 18.41	ns	ns
Placebo	80.69 ± 44.02	108.03 ± 57.50	ns	
LDH (u/l)				
Spirulina	396.53 ± 43.03	316.86 ± 37.74	ns	<0.001**
Placebo	375.45 ± 50.49	399.31 ± 98.30	ns	
LA (mg/dl)				
Spirulina	20.40 ± 3.01	45.57 ± 5.04	0.001**	ns
Placebo	27.29 ± 3.16	33.01 ± 5.85	ns	
PH of urine				
Spirulina	6.75 ± 0.30	5.75 ± 0.30	ns	ns
Placebo	6.25 ± 0.41	6.13 ± 0.34	ns	
RQ				
Spirulina	1.14 ± 0.04	1.11 ± 0.02	ns	ns
Placebo	1.13 ± 0.02	1.11 ± 0.03	ns	
TE (s)				
Spirulina	713 ± 40.40	765 ± 36.30	0.014*	ns
Placebo	720 ± 62.96	743 ± 60.41	ns	

Values for each group represent mean ± SE ($n = 8$). *Sb* × *Sa* a paired Student's *t*-test for comparing the mean difference between before and after test of spirulina supplementation, *Pb* × *Pa* a paired Student's *t*-test for comparing the mean difference between before and after test of placebo supplementation, *T* × *S* the interaction effect of time and supplementation with the ANCOVA test, *ns* = no significant different

Value in the *Sb* × *Sa*, *Pb* × *Pa* or *T* × *S* column means the significant probability

*Significant difference ($P < 0.05$)

**Highly significant ($P < 0.01$)

Each individual of pre-supplementation (range from 13.73 to 136.19 u/l in the spirulina group and from 1.21 to 380.54 u/l in the placebo group) and post-supplementation (range from 5.53 to 174.59 u/l in the spirulina group and from 8.89 to 504.48 u/l in the placebo group) on CK parameter showed large deviation in their figures leaded to the average within and between these two groups all showed with no significant difference.

The TE recorded during all-out treadmill exercise is significantly raised from 713 to 765 s after supplementation with spirulina by pair *t*-test. Even the TE mean value was also raised from 720 to 743 s; there is no significant difference within the soy protein group (Fig. 1). The ANCOVA test of TE variable showed no significant difference.

Discussion

The aim of this study is to verify the effect of eating *S. platensis* on protecting skeletal muscle damage under oxidative stress, which was induced by exhaustive exercise. Many studies about oxidative stress during highly strength training or competition were done. However, the type and strength to form oxidative stress by exercise in many published researches were quite different. Those treatments were designed such as muscular breakdown following the marathon in human (Langberg et al. 2000), 7-day eccentric training period on human muscle damage (Chen and Hsieh 2001), membrane leakage and increased content of Na^+ -K^+ pumps and Ca^{2+} in human muscle after a 100-km run (Overgaard et al. 2002), muscle damage after endurance exercise of the elbow flexors in human (Nosaka et al. 2002), myocardial injury following treadmill exercise testing in patients (Ashmaig et al. 2001), myocardium injury following swimming in rats (Chen et al. 2000), and myocardium injury following treadmill running program in canine (Stuewe et al. 2001), etc. In the current study, we used Bruce protocol as exercise stimulus could have two benefits. One is on laboratory standardizing. Another is on the experimental ethical. The skeleton muscle was not damaged after one exercise stimulus by all-out protocol (Table 2). The exercise prescription is ethical on the aspect of exercise intensity.

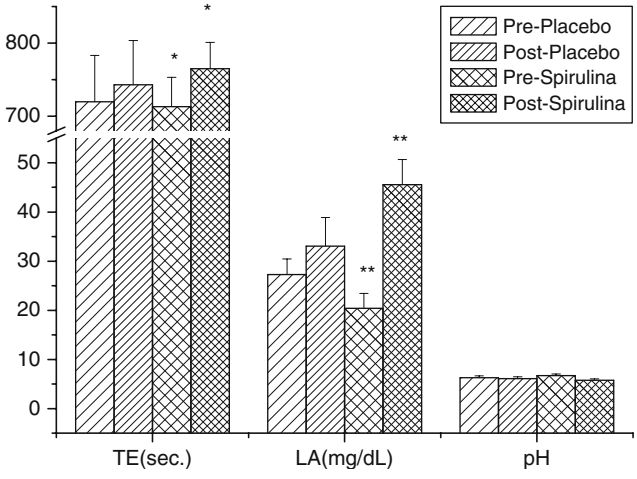

Fig. 1 Changes in TE, serum LA, and ureic pH before and after 3 weeks of placebo (15 g soy protein/day) or spirulina (15 g/day) treatment. Values represent mean ± SE. *Significant difference ($P < 0.05$) between pre- and post-trial. **Highly significant difference ($P < 0.01$) between pre- and post-trial

For eliminating the psychology factor from participants, it is necessary to give a placebo on the experimental design of double blind. Concerning the high-protein contain of spirulina, we chose the soy protein as controlling placebo. The results of this research showed soy protein has the effect of anti-oxidation as studied before (Song et al. 1996). Soy protein in this study probably could be looked as another supplementation other than placebo that is better. This is the reason why we decided to use the ANCOVA rather than ANOVA statistics analysis.

It was well established that the exercise induced increase in oxygen consumption in tissues results in an increased production of free radicals and the failure to remove free radicals could lead to oxidative damage of cellular biomolecules, including cell membrane biomolecules, cytoslic biomolecules, even to the cell nucleus biomolecules DNA (Jackson 1998). The MDA, a metabolite of phospholipid per-oxidation, is a popular index of first condition on living body oxidative damage (Jain 1988). The current study showed that the concentrations of plasma MDA were significantly decreased from 56.21 to 50.37 nmol/ml after supplementation with spirulina. There was less increase of plasma MDA from 54.31 to 55.08 nmol/ml after supplementation with soy protein (Table 2). However, there was no significant difference between supplementation with soy protein and spirulina by an ANCOVA analysis. As per the above findings, it is suggested that after loaded with exhaustive exercise, human supplementation spirulina have the effect of reducing lipid per-oxidation. The changes of MDA levels before and after spirulina supplementation on the current human-study results is similar to those of the previous published animal-study by Huang et al. (2000).

In normal condition, per-oxidation injuries will promote anti-oxidation adaptation within human body. SOD and GP_x are two different anti-oxidation enzymes

major in the cytosol of living cells. We measured these two enzymes from red blood cell following the protocols of Randox, respectively. There are two different kinds of count unit in SOD and GP_x. One is u/l and the other is u/gHb. Athletes always have large different range of red blood cell number, depending mainly on the extent of oxidation injuries or even exercise-anemia. The u/gHb unit could better reflect real activity of these enzymes on the athletes. We standardized the u/gHb unit by measuring the hemoglobin concentrations of each athlete (data not shown) by the protocols of Randox (hemoglobin, Randox kit: HG1539) rather than the mean value of hemoglobin of human normal value.

Song et al. (1996) found that soy protein diet increases hepatic SOD activity, while endurance exercise training is effective in increasing hepatic SOD activity on a normal protein diet in rats. Huang et al. (2000) concluded that *S. platensis* supplement decreased free radicals and increased soleus SOD activities significantly in rats after exhaustive exercise. Our studies show that the concentration of blood SOD is significantly raised from 1,324.09 to 1,852.45 u/gHb after supplementation with spirulina. The concentration of blood SOD is also significantly raised from 1,251.10 to 1,510.10 u/gHb after supplementation with soy protein. However, there is no significant difference between supplementation with soy protein and spirulina by an ANCOVA analysis. In other words, human supplementation spirulina or soy protein has the effect of inducing blood SOD activity after endurance exercise, but there is no significant difference on the blood SOD level between supplementation with spirulina or soy protein.

Song et al. (1996) concluded that significant increases hepatic GP_x activity in the soy protein groups was observed when rats were loaded with endurance exercise training. However, there were no GP_x parameter that could be found in any of publishes, which experiment design with spirulina supplementation, after any kind of exercise stimulus. Our studies showed that the concentration of blood GP_x is not significantly raised from 29.01 to 33.86 u/gHb and 21.32 to 22.15 u/gHb after supplementation with spirulina and soy protein, respectively. However, there was significant difference with the probability of 0.018 between supplementation with soy protein and spirulina by an ANCOVA analysis. Our results show that human supplementation with spirulina has significant increasing blood GP_x level than supplementation with soy protein.

Serum concentrations of CK (S-CK) are usually measured as an indicator of muscular breakdown in response to the exercise bout (Langberg et al. 2000) or low-intensity continuous muscle contractions (Nosaka et al. 2002). The normal range of CK in serum is 24–195 u/l for men and 24–170 u/l for women (Langberg et al. 2000). The tested participants showed their CK activities before the supplementation treatment, which showed a wide range of 2.1–380.54 u/l. Although the average of CK activities between the tested groups showed large difference, they were insignificant statistically. The differences of CK activities before and after supplementation treatment were thus to represent the increase or decrease effects of spirulina or soy protein on CK activities. Our result showed that the average of CK activity in the group having spirulina appeared to decrease 28.77% after 3 weeks of the experiment period, while there was a 33.88% increase of CK concentration in the soy protein group. Taking spirulina as food supplement 7.5 mg daily seemed to have protected the skeletal muscle from damage after strenuous exercise.

Lactate dehydrogenase, which is an enzyme mainly in the sarcoplasm of skeletal muscle, is responsible for the removal of LA produced after fast anaerobic consumption of glucose due to muscle contraction. In normal condition, the activity of serum LDH reflects the degree of body LA metabolism and the extent of basal cell damage. The normal range of LDH in serum is 230–460 u/l for adult and with no difference between the genders (Randox protocols). An excess activity of serum LDH may represent a leak of enzyme from the damaged muscle cells due to per-oxidative injure, crash, or other symptoms of diseases. However, individuals involved in this research had their distinct values falling in or out of the normal range upto 969.84 u/l (data not shown), and they were insignificant statistically between groups. Even with such a great difference among individuals, food supplement of spirulina in this research gave a decrease of 20.09% on average of LDH activity after 3 weeks of the experiment period. On the contrary, the participants in the soy protein group showed little increase of average activity after 3 weeks of the experiment period. Furthermore, there was significant difference with the probability of less than 0.001 between supplementation with soy protein and spirulina by ANCOVA analysis. These results suggest that taking spirulina as food supplementation had a tendency to decrease the blood concentration of LDH.

High-oxygen content in tissues or organs resulting from rapid breath may lead to the formation of free radicals that will make fragile the cell membrane by forming lipid peroxide (Alessio et al. 2000). As a consequence, an athlete gets the injury by the effect of per-oxidation during training or competition. In this study,

we used the CK and LDH as makers for skeletal muscle damage. The data of all our results did not entirely show statistic significance by unpaired Student's *t*-tests. However, it probably can explain the effect by the tendency of mean value and ANCOVA test between before and after diet treatment on each variable. We suggest that the blue–green algae or cyanobacteria, *S. platensis*, taken as food supplement, showed preventive effect on the skeletal muscle from the exercise per-oxidation injury probably by reducing the pre-oxidative level in muscle during exhaustive exercise. These results are similar to the one reported by Huang et al. (2000), which concluded that *S. platensis* could exert a protective effect from exercise-induced muscle damage in rats based on the decrease of serum CK and LDH level.

While an athlete is giving full play to his ability in a contest, his skeletal muscle and circulation system are in their utmost situation on oxygen utility or transportation. Between these two systems, one is responsible for the intensive body motions by energy metabolism formatting the ATP and the other one is supporting the blood stream in offering air oxygen to the needing tissues. High-oxygen content in skeletal muscle and circulation system resulting from rapid breath may lead to the formation of free radicals that will make fragile the cell membrane by forming lipid peroxide (Jackson 1998; Alessio et al. 2000; Mastaloudis et al. 2001). Excessive free radicals or reactive oxygen species has been shown to have an adverse effect on oxidative injuries and muscle fatigue, and will influence the function of skeletal muscle contractile leading to exert a negative impact on performance (Powers et al. 2004). It deserves to be mentioned that the time of all-out by Bruce protocol in this study recorded is significantly raised from 713 to 765 s after supplementation with spirulina and showed no significant difference within soy protein group (Fig. 1), although all-out exercise before blood drawn used was an inducing factor for oxidative stress. As with the Zhang and Liu (1999) report, polysaccharide from *S. platensis* is a main bioactive anti-fatigue material and could prolong the moving endurance time on mice. The findings of current study on the endurance performance are exciting, although the participants were untrained humans.

The tendency of pH value on urine was down after supplementation with spirulina, although statistical analysis was not significant. This result was out of expectation because the high-alkalinity characteristic of spirulina should have the effect of reduction in our body. However, there are evidences of both TE and LA variables to be able to explain the pH tendency. The TE was postponed and the LA concentration was highly significantly increasing on the spirulina trail (Fig. 1). It is the reason why the tendency of pH value on urine was unexpected after supplementation with spirulina. The result of pH tendency and LA variable combined with the TE variable could probably provide evidence supplementation of spirulina has the effect on endurance exercise.

This study is to directly verify the effect on protecting skeletal muscle damage from the oxidative stress and unwittingly found the effect on postponement of the TE during treadmill exercise by eating *S. platensis*. Spirulina is a nature food other than artificial concentrated compounds. If taking spirulina has the effect on exercise related advantage, it could probably decrease the side effect and dependence, which usually appear with long-term use of artificial concentrated compounds on human body.

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