Ergogenic and Antioxidant Effects of Spirulina Supplementation in Humans

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BASIC SCIENCES

ABSTRACT

KALAFATI, M., A. Z. JAMURTAS, M. G. NIKOLAIDIS, V. PASCHALIS, A. A. THEODOROU, G. K. SAKELLARIOU, Y. KOUTEDAKIS, and D. KOURETAS. Ergogenic and Antioxidant Effects of Spirulina Supplementation in Humans. *Med. Sci. Sports Exerc.*, Vol. 42, No. 1, pp. 142–151, 2010. **Purpose**: Spirulina is a popular nutritional supplement that is accompanied by claiMSS for antioxidant and performance-enhancing effects. Therefore, the aim of the present study was to examine the effect of spirulina supplementation on (i) exercise performance, (ii) substrate metabolism, and (iii) blood redox status both at rest and after exercise. **Methods**: Nine moderately trained males took part in a double-blind, placebo-controlled, counterbalanced crossover study. Each subject received either spirulina (6 $g \cdot d^{-1}$) or placebo for 4 wk. Each subject ran on a treadmill at an intensity corresponding to 70%–75% of their $\dot{V}O_{2max}$ for 2 h and then at 95% $\dot{V}O_{2max}$ to exhaustion. Exercise performance and respiratory quotient during exercise were measured after both placebo and spirulina supplementation. Blood samples were drawn before, immediately after, and at 1, 24, and 48 h after exercise. Reduced glutathione (GSH), oxidized glutathione (GSSG), GSH/GSSG, thiobarbituric acid-reactive substances (TBARS), protein carbonyls, catalase activity, and total antioxidant capacity (TAC) were determined. **Results**: Time to fatigue after the 2-h run was significantly longer after spirulina supplementation (2.05 ± 0.68 vs 2.70 ± 0.79 min). Ingestion of spirulina significantly decreased carbohydrate oxidation rate by 10.3% and increased fat oxidation rate by 10.9% during the 2-h run compared with the placebo trial. GSH levels were higher after the spirulina supplementation compared with placebo at rest and 24 h after exercise. TBARS levels increased after exercise after placebo but not after spirulina supplementation. Protein carbonyls, catalase, and TAC levels increased similarly immediately after and 1 h after exercise in both groups. **Conclusions**: Spirulina supplementation induced a significant increase in exercise performance, fat oxidation, and GSH concentration and attenuated the exercise-induced increase in lipid peroxidation. **Key Words:** FREE RADICALS, REACTIVE OXYGEN SPECIES, REDOX STATUS, OXIDATIVE STRESS, PHYSICAL ACTIVITY

Spirulina (*Spirulina platensis*) is a photosynthetic cyanobacterium that possesses biological activity and is widely cultivated to produce nutritional supplements (26). Spirulina is rich in essential amino acids and fatty acids (palmitic acid, linoleic acid, and γ-linolenic acid), vitamin C, vitamin E, and selenium (26). Recently, attention has been placed on the antioxidant potential of spirulina. Indeed, many of the chemical components of spirulina, such as phenolic compounds, tocopherols, β-carotenes, and phycocyanins exhibit antioxidants properties (11). For instance, it has been reported that spirulina supplementation with ginseng decreased lipid peroxidation and increased the levels of reduced glutathione (GSH), superoxide dismutase, and glutathione peroxidase in the kidney of rats (21).

Exercise promotes the production of reactive oxygen and nitrogen species (RONS). Growing evidence indicates that RONS contribute to muscle fatigue (14). To protect against exercise-induced oxidative damage, cells contain endogenous cellular defense mechanisMSS to control the levels of RONS (37). Furthermore, exogenous dietary antioxidants interact with endogenous antioxidants and form a network of cellular antioxidants (37). The fact that exercise-induced RONS production can contribute to muscle fatigue (14) has resulted in numerous investigations examining the effects of different antioxidants (e.g., vitamin C or *N*-acetylcysteine) on human redox status and exercise performance (e.g., [2,28]). However, comparatively few researchers have studied the effect of foods rich in antioxidants on oxidative stress provoked by exercise (34,45). Thus, the extent to

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which foods rich in antioxidants (such as spirulina) modify the redox status responses induced by exercise is largely unknown.

We found only one study that examined the effects of spirulina on redox status and exercise performance (25). However, the blood samples after spirulina supplementation and exercise were compared with the resting blood samples making it difficult to discern the spirulina effect. Nowadays, spirulina is a very popular nutritional supplement for humans and is accompanied by claiMSS for antioxidant and performance-enhancing effects (12). These claiMSS are extrapolated by the findings of *in vitro* and animal studies (11,21) but have not been substantiated concerning humans. Therefore, the aim of the present study was to examine the effect of spirulina supplementation on (i) exercise performance, (ii) substrate metabolism, and (iii) blood redox status both at rest and after exercise.

MATERIALS AND METHODS

Subjects. Nine healthy moderately trained men (age = 23.3 ± 1.7 yr, height = 174.3 ± 1.7 cm, weight = 70.7 ± 1.9 kg, body fat = 9.8 ± 1.3%, maximal oxygen consumption ($\dot{V}O_{2max}$) = 52.2 ± 1.8 mL·kg^{-1}·min^{-1}) volunteered to participate. The subjects were recreational runners and had trained for at least 1 yr (3.4 ± 1.1 yr), at least two times per week (3.1 ± 0.9 times per week), at least 45 min per session (56 ± 10 min per session). All subjects were informed thoroughly about the risks, the possible discomforts, and the benefits of the study before signing a written informed consent. All subjects completed a medical and supplementation history and physical activity questionnaire to determine eligibility. No subject was a smoker or taking supplements or anti-inflammatory drugs. The procedures were in accordance with the Helsinki Declaration of 1975 and approved by the institutional review board.

Baseline measurements. One to two weeks before the first exercise trial, subjects visited the laboratory for baseline measurements. Body mass was measured to the nearest 0.5 kg with subjects lightly dressed and barefoot (Beam Balance 710; Seca, Birmingham, United Kingdom) and standing height was measured to the nearest 0.5 cm (Stadiometer 208; Seca). Percentage body fat was calculated from seven skinfold measurements using a Harpenden skinfold caliper (John Bull, British Indicators Ltd, St. Albans, United Kingdom) according to published guidelines (4). To establish that all subjects ran at similar exercise intensity, $\dot{V}O_{2max}$ was determined using a treadmill test to exhaustion. The protocol began at 10 km·h^{-1} and was increased by 1 km every 2 min until $\dot{V}O_{2max}$ was reached. $\dot{V}O_{2max}$ test was terminated when three of the following four criteria were met: (i) subject exhaustion, (ii) a <2 mL·kg^{-1}·min^{-1} increase in $\dot{V}O_2$ with an increase in work rate, (iii) a respiratory exchange ratio ≥1.10, and (iv) an HR within 10 bpm of the theoretical maximum HR (220 − age). Respiratory gas variables were measured using a metabolic cart (Vmax29; SensorMedics, Yorba Linda, CA), which was calibrated before each test using standard gases of known concentration. Exercise HR was monitored by telemetry (Tester S610™; Polar, Electro Oy, Finland).

Study design. A double-blind, placebo-controlled, counterbalanced crossover design was used (i.e., half of the subjects were given the spirulina first and the other half were given the placebo and the reversed). Each subject participated in four exercise trials (Fig. 1). In the first exercise trial, subjects visited the laboratory 7–14 d after

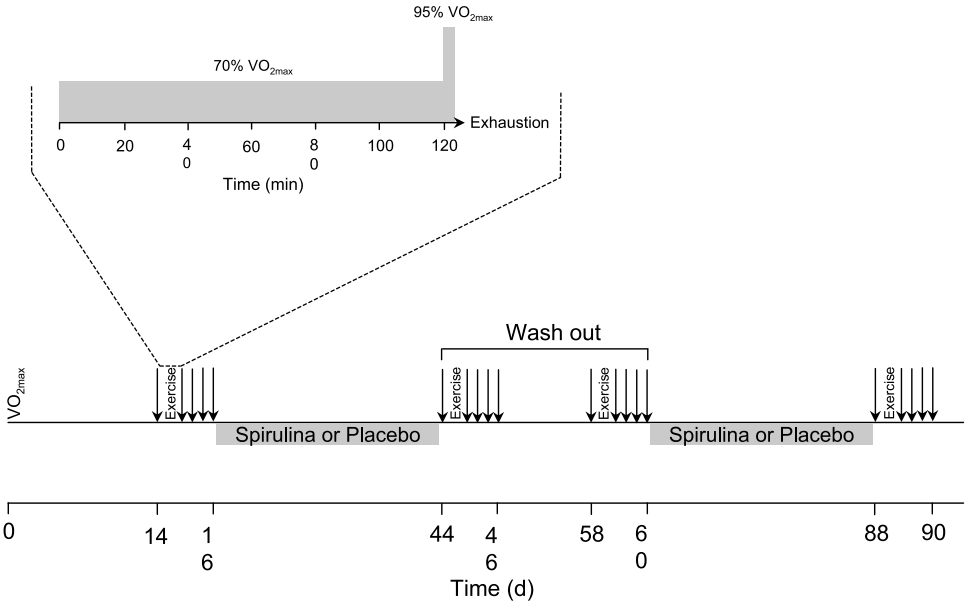

FIGURE 1—Study design. ***Arrows*** **indicate blood sampling.**

$\dot{V}O_{2max}$ determination (between 08:00 and 10:00), where they ran on a treadmill at an intensity corresponding to 70%–75% of their $\dot{V}O_{2max}$ for 2 h. After the 2-h run, the speed of the treadmill was increased to elicit the 95% $\dot{V}O_{2max}$, and exercise was terminated at exhaustion (31). Fatigue was considered to have occurred when the required speed could not be maintained by the subject or when the subject stopped voluntarily. The time to reach volitional fatigue was recorded and used as an index of aerobic performance. Expired gas samples were obtained every 10 min to ensure the prescribed exercise intensity and to calculate the fat and carbohydrate oxidation rates. Water (250 mL) was given to the volunteers every 20 min during exercise. After the end of the initial exercise trial, each subject consumed two capsules (1 g each) containing either *S. platensis* manufactured by Algae AC (Serres, Greece) or 100% egg protein (placebo). The capsules were consumed before meals three times per day for 4 wk. The daily dosage of spirulina that was used (6 $g \cdot d^{-1}$) was close to other relevant human studies (7.5 [15] and 8 [25] $g \cdot d^{-1}$). One day after the end of the 4-wk supplementation period, subjects came back to the laboratory to perform the second exercise bout with identical conditions as the first exercise trial. A 2-wk washout period occurred between the second and the third exercise trials to avoid possible carryover effects. After the washout period, the subjects came back for a third and fourth times, where the exercise conditions of the first and second exercise trials were followed. The first and third exercise trials were performed to ensure that the 2-wk washout period was adequate to have similar physiological and biochemical values before the two periods of supplementation. We are aware of only one study that investigated the effects of spirulina supplementation on humans using a crossover design (6). In this study, a 2-wk washout period was also used. In addition, taking into account the short supplementation period used in the present study (i.e., 4 wk), we considered that the 2-wk washout period would be long enough for any effects of placebo or spirulina to disappear.

The basic composition of dry spirulina is as follows: 63.3% protein, 7.1% lipid, and 15.2% carbohydrate (50), 101 mg of vitamin C (5), 15 mg of vitamin E, and 0.13 mg of selenium per 100 g (50), as well as 43.6% palmitic acid, 17.2% linoleic acid, and 21.7% γ-linolenic acid of total fatty acids (33).

Fat and carbohydrate oxidation. Fat and carbohydrate oxidation rates ($g \cdot min^{-1}$) were calculated indirectly by monitoring the rate of O_2 consumption ($L \cdot min^{-1}$) and CO_2 production ($L \cdot min^{-1}$) using the following stoichiometric equations (18), assuming that protein oxidation during exercise was negligible:

$$\text{fat oxidation} = 1.695\dot{V}O_2 - 1.701\dot{V}CO_2$$
$$\text{carbohydrate oxidation} = 4.210\dot{V}CO_2 - 2.962\dot{V}O_2$$

Blood collection and handling. Blood samples were drawn from a forearm vein at rest and after exercise (immediately after exercise and at 1, 24, and 48 h after exercise). Directly after taking the blood sample, 0.5 mL of blood was placed in a tube containing EDTA for the determination of hematocrit and hemoglobin. Whole-blood lysate was produced by adding 5% trichloroacetic acid (TCA) to whole blood (1:1 v/v) collected in EDTA tubes for reduced GSH and oxidized glutathione (GSSG) analysis. The whole-blood samples were centrifuged at 4000*g* for 10 min at 4°C, and the supernatant was removed and centrifuged again at 28,000*g* for 5 min at 4°C. The clear supernatant was collected in Eppendorf tubes and stored at −80°C until GSH and GSSG determination. Another portion of blood was collected in plain tubes, left on ice for 20 min to clot, and centrifuged at 1500*g* for 10 min at 4°C for serum separation. Serum was transferred in Eppendorf tubes and was used for the determination of creatine kinase, thiobarbituric acid-reactive substances (TBARS), protein carbonyls, catalase, and total antioxidant capacity (TAC). Serum samples were stored in multiple aliquots at −80°C and were thawed only once before analysis.

Assays. A slightly modified version of Reddy et al. (40) was used to measure GSH, which is originally based on Beutler et al. (7). Twenty microliters of whole blood treated with TCA was mixed with 660 μL of 67 mM sodium potassium phosphate (pH 8.0) and 330 μL of 1 mM 5,5-dithiobis-2-nitrobenzoate (DTNB). The samples were incubated in the dark at room temperature for 45 min, and the absorbance was read at 412 nm. A standard curve was constructed by using GSH as a standard at concentrations of 0, 0.25, 0.50, and 1 mM. GSSG was determined according to Tietze (49). Two hundred and sixty microliters of whole blood treated with TCA was neutralized up to pH 7.0–7.5 with NaOH. Four microliters of 2-vinyl pyridine was added, and the samples were incubated for 2 h at room temperature. Five microliters of whole blood treated with TCA was mixed with 600 μL of 143 mM sodium phosphate (6.3 mM EDTA, pH 7.5), 100 μL of 3 mM nicotinamide dinucleotide phosphate (NADPH), 100 μL of 10 mM DTNB, and 194 μL of distilled water. The samples were incubated for 10 min at room temperature. After the addition of 1 μL of glutathione reductase, the change in absorbance at 412 nm was read for 3 min. A standard curve was constructed by using GSSG as a standard at concentrations of 0, 0.025, 0.050, and 0.100 mM. The GSH/GSSG ratio was calculated for each subject, and the means of these ratios for each time point are presented.

TBARS were measured according to Keles et al. (22). One hundred microliters of serum was mixed with 500 μL of 35% TCA and 500 μL of Tris–HCl (200 mM, pH 7.4) and incubated for 10 min at room temperature. One microliter of 2 M Na_2SO_4 and 55 mM thiobarbituric acid solution was added, and the samples were incubated at 95°C for 45 min. The samples were cooled on ice for 5 min and were vortexed after adding 1 mL of 70% TCA. Finally, the samples were centrifuged at 15,000*g* for 3 min, and the absorbance of the

supernatant was read at 530 nm. A standard curve was constructed by using malondialdehyde as a standard at concentrations of 0, 1.25, 2.5, 5, and 10 μM.

Protein carbonyls were measured according to Patsoukis et al. (36). In 50 μL of serum, 50 μL of 20% TCA was added, incubated in the ice bath for 15 min, and centrifuged at 15,000*g* for 5 min at 4°C. The supernatant was discarded, and 500 μL of 10 mM 2,4-dinitrophenylhydrazine (in 2.5N HCl) for the sample, or 500 μL of 2.5N HCl for the blank, was added to the pellet. The samples were incubated in the dark at room temperature for 1 h, with intermittent vortexing every 15 min, and were centrifuged at 15,000*g* for 5 min at 4°C. The supernatant was discarded, and 1 mL of 10% TCA was added, vortexed, and centrifuged at 15,000*g* for 5 min at 4°C. The supernatant was discarded, and 1 mL of ethanol–ethyl acetate (1:1 v/v) was added, vortexed, and centrifuged at 15,000*g* for 5 min at 4°C. The washing step was repeated two more times. The supernatant was discarded, and 1 mL of 5 M urea (pH 2.3) was added, vortexed, and incubated at 37°C for 15 min. The samples were centrifuged at 15,000*g* for 3 min at 4°C, and the absorbance was read at 375 nm. Protein carbonyls values were obtained by using the extinction coefficient of 2,4-dinitrophenylhydrazine (22 $mM \cdot cm^{-1}$).

Catalase activity was measured according to Aebi (1). In 20 μL of serum, 2975 μL of 67 mM sodium potassium phosphate (pH 7.4) was added, and the samples were incubated at 37°C for 10 min. Five microliters of 30% hydrogen peroxide was added to the samples, and the change in absorbance was immediately read at 240 nm for 1.5 min. Catalase activity was obtained by using the extinction coefficient of hydrogen peroxide (43.6 $M \cdot cm^{-1}$).

TAC was measured according to Janaszewska and Bartosz (17). For TAC, in 20 μL of serum, 480 μL of 10 mM sodium potassium phosphate (pH 7.4) and 500 μL of 0.1 mM 2,2-diphenyl-1 picrylhydrazyl (DPPH) were added and incubated in the dark for 30 min at room temperature. The samples were centrifuged for 3 min at 20,000*g*, and the absorbance was read at 520 nm. TAC values were obtained by calculating the number of DPPH molecules scavenged per minute.

Serum creatine kinase was determined spectrophotometrically using a commercially available kit (Spinreact, Sant Esteve, Spain). Total protein in serum was assayed using a Bradford reagent. Postexercise plasma volume changes were computed based on hematocrit and hemoglobin. Hematocrit was measured by microcentrifugation, and hemoglobin was measured using a kit from Spinreact. Each assay was performed in duplicates, except for GSSG, which was performed in triplicates. The intra-assay coefficient of variation for each measurement was as follows: GSH 4.0%, GSSG 6.5%, TBARS 3.9%, protein carbonyls 5.5%, catalase 6.7%, TAC 3.7%, and creatine kinase 2.9%.

Dietary analysis. To factor the effect of the diet on the outcome measures of the study and to establish that participants had similar levels of macronutrient and antioxidant intake during the period of data collection, they were asked to record their diet for 3 d preceding their first visit to the laboratory and to repeat this diet before their next three visits to the laboratory. Each subject had been provided with a written set of guidelines for monitoring dietary consumption and a record sheet for recording food intake. Diet records were analyzed using the nutritional analysis system ScienceFit Diet 200A (ScienceFit, Athens, Greece).

Statistical analysis. The distribution of all dependent variables was examined by the Shapiro–Wilk test and was found not to differ significantly from normal. First, to ensure that the 2-wk washout period was adequate, the data from the first and the third trials were analyzed through two-way (trial × time) ANOVA with repeated measures on time. Second, to evaluate the effects of supplementation and exercise, the data from the second and the fourth trials were analyzed through two-way (group × time) ANOVA with repeated measures on time. If a significant interaction was obtained, pairwise comparisons were performed through simple main effect analysis. Differences in diet among trials or groups were examined through one-way ANOVA. Aerobic performance at the second and fourth exercise trials was examined by paired *t*-test. Carbohydrate and lipid oxidation rates during the 2-h run at the second and fourth exercise trials were also examined by paired *t*-test. Statistical significance was considered when $P < 0.05$. The SPSS version 15.0 was used for all analyses (SPSS, Inc., Chicago, IL). Data are presented as mean ± SEM.

RESULTS

Washout, compliance, and diet. The comparison of the data from the first and the third trials revealed no significant interaction and no significant main effect of trial on any of the dependent variables measured. Therefore, the 2-wk washout period proved adequate to have similar physiological and biochemical values before the two periods of supplementation. Supplementation compliance was 97.6% and 96.4% for placebo and spirulina, respectively, as revealed by the counting of the capsules provided upon return of the bottles. No adverse effects were reported after spirulina supplementation. Dietary intake, assessed during the 3-d period, showed no differences between groups in any of the assessed variables (Table 1).

Exercise performance. The average exercise intensity during the 2-h submaximal run for the placebo and spirulina trials was 70.6 ± 2.4% and 71.0 ± 1.9 % of $\dot{V}O_{2max}$, respectively ($P > 0.05$). Time to fatigue after the 2-h run was significantly higher after spirulina supplementation (2.05 ± 0.68 vs 2.70 ± 0.79 min for the placebo and spirulina groups, respectively, $P = 0.048$; Fig. 2). Time to fatigue at 95% $\dot{V}O_{2max}$ was reproducible in preliminary trials (coefficient of variance (CV) 6.2 ± 0.7%).

TABLE 1. Analysis of daily energy intake after placebo and spirulina supplementation (mean ± SEM).

	Placebo	Spirulina
Energy (kcal)	2537 ± 127	2421 ± 61
Carbohydrate (% energy)	44.0 ± 2.8	47.0 ± 1.7
Fat (% energy)	39.0 ± 3.1	37.1 ± 2.9
Protein (% energy)	17.0 ± 1.3	15.9 ± 1.6
Vitamin A (mg, RE)	999 ± 164	663 ± 177
Vitamin C (mg)	169 ± 18	162 ± 18
Vitamin E (mg, α-TE)	11.0 ± 1.4	11.5 ± 1.3
Selenium (μg)	155 ± 11	123 ± 5

α-TE, α-tocopherol equivalents; RE, retinol equivalents.

Fat and carbohydrate oxidation. Supplementation of spirulina significantly decreased carbohydrate oxidation rate by 10.3% ($P = 0.008$) and increased fat oxidation rate ($P = 0.003$) by 10.9% during the 2-h run compared with the placebo trial (Fig. 3).

Plasma volume. Plasma volume did not change during the 48-h postexercise period in both groups ($P > 0.05$); nevertheless, the values were corrected for any nonsignificant plasma volume changes.

Creatine kinase. There was no significant main effect of group or time × group interaction concerning serum creatine kinase (Fig. 4). However, there was a significant main effect of time ($P < 0.001$), with creatine kinase activity increasing 24 and 48 h after exercise in both groups.

GSH status. There was no significant main effect of time or group × time interaction concerning GSH (Fig. 5A). However, there was a significant main effect of group ($P = 0.049$), with GSH level being higher after the spirulina supplementation at rest and 24 h after exercise. There were no significant main effects or interactions for GSSG and GSH/GSSG ratio (Figs. 5B and C).

TBARS and protein carbonyls. There was no significant main effect of group or time concerning serum TBARS (Fig. 6A). However, there was a significant group × time interaction ($P = 0.007$), with TBARS levels increasing after exercise after placebo but not after spirulina supplementation. There was no significant main effect of group or time × group interaction concerning serum protein carbonyls (Fig. 6B). However, there was a significant main effect of time ($P < 0.001$), with protein carbonyls levels increasing immediately after and 1 h after exercise in both groups.

Catalase and TAC. There was no significant main effect of group or group × time interaction concerning serum catalase (Fig. 7A). However, there was a significant main effect of time ($P < 0.001$), with catalase activity increasing immediately after and 1 h after exercise in both groups. There was no significant main effect of group or group × time interaction concerning serum TAC (Fig. 7B). However, there was a significant main effect of time ($P < 0.001$), with TAC increasing immediately after and 1 h after exercise in both groups.

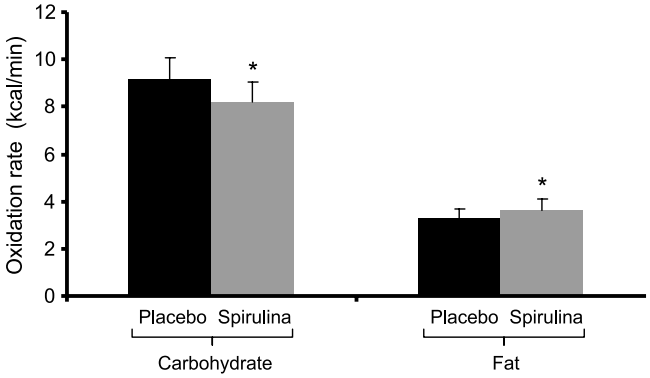

FIGURE 3—Oxidation rate in the placebo and spirulina trial during the 2-h run (mean ± SEM). *The carbohydrate and fat rates were significantly different between the placebo and the spirulina trials ($P < 0.05$).

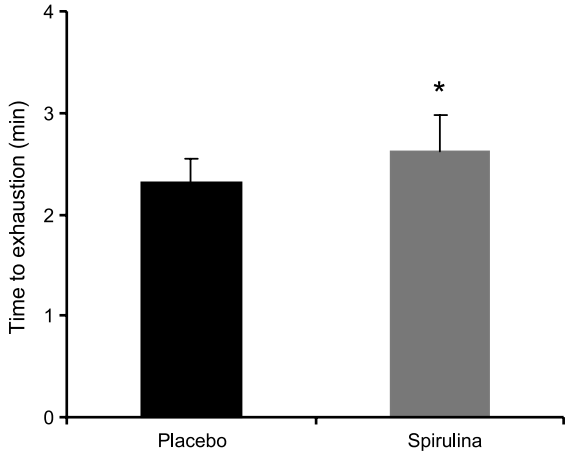

FIGURE 2—Exercise performance at 95% $\dot{V}O_{2max}$ in the placebo and spirulina trial (mean ± SEM). *Significantly different from the placebo trial ($P < 0.05$).

DISCUSSION

To our knowledge, this is the first attempt to examine the effects of spirulina supplementation on exercise performance,

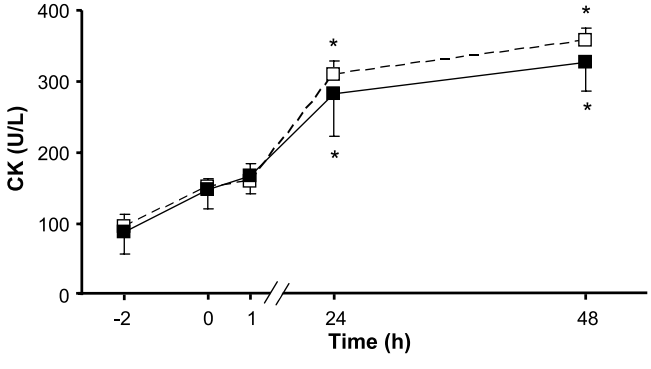

FIGURE 4—Creatine kinase (CK) activity in the placebo (*open rectangles*) and spirulina exercise trials (*filled rectangles*; mean ± SEM). *Significantly different from the resting value in the same trial ($P < 0.05$).

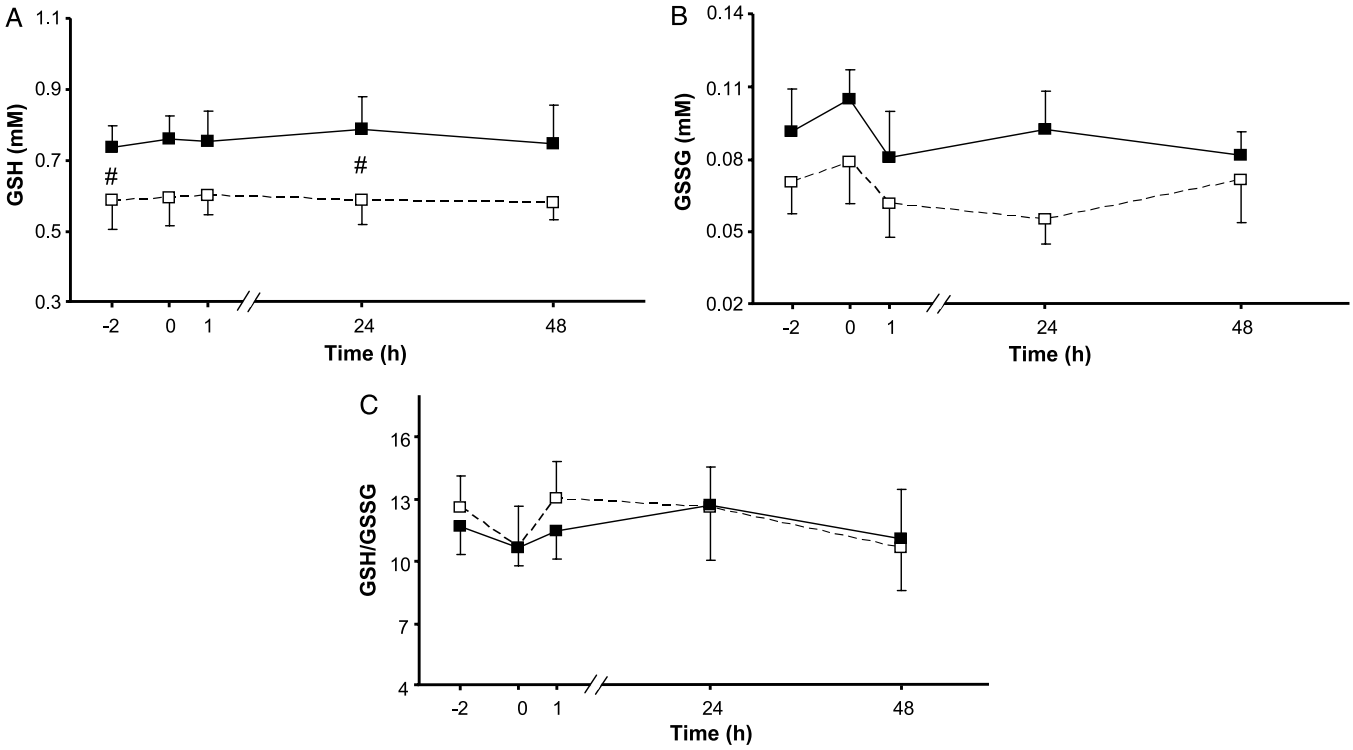

FIGURE 5—GSH (*A*) and GSSG concentrations (*B*) as well as GSH/GSSG (*C*) ratio in the placebo (*open rectangles*) and spirulina exercise trials (*filled rectangles*; mean ± SEM). #Significantly different between placebo and spirulina trial at the same time point ($P < 0.05$).

substrate metabolism, and blood redox status at rest and after exercise in humans. The results showed that spirulina supplementation for 4 wk induced a significant increase in exercise performance, fat oxidation, and glutathione concentration as well as attenuated exercise-induced increases in lipid peroxidation. This provides evidence that increased levels of fat oxidation and GSH may contribute to enhanced exercise performance.

Exercise performance and increased fat oxidation rate. Probably the most interesting finding of the present study is the increase in exercise performance after spirulina supplementation. Despite the fact that the mechanism behind the ergogenic effect of spirulina is difficult to be identified, the most plausible explanation implicates fat oxidation, the rate of which was found substantially increased (15.8%) during the 2-h exercise trial in spirulina-supplemented individuals. The maintenance of maximal aerobic power output requires that carbohydrates are oxidized as well as fats (15). Because carbohydrates come from the glycogen stores, the time that maximal aerobic power can be sustained depends on the amount of glycogen stored initially (15). In fact, it was found that the time to exhaustion when working at 75% of maximal aerobic power (almost equal to 70% $\dot{V}O_{2max}$ that was used in the present study) correlated with the initial muscle glycogen concentration (15). Moreover, there is evidence that increasing fat oxidation leads to sparing of glycogen (15); thus, at least in principle, the increased fat oxidation could have spared glycogen or glucose to allow high-intensity exercise to be continued for a longer time.

We have no hint as to what biochemical mechanism may have led to increased fat oxidation after spirulina supplementation, partly because spirulina is a complex mixture of substances with different properties. Potential control points of fat oxidation include lipolysis in adipose tissue, transportation of fatty acids via blood, transportation of fatty acids to muscle, hydrolysis of myocellular triacylglycerols, transportation of fatty acids to mitochondria, and mitochondrial density (30). We know very little about whether and how spirulina affects these processes. However, the high content of γ-linolenic acid in spirulina (21.7% of total fatty acids in dry spirulina [33]) may play a role in mediating the reported effects on fat metabolism in the present study. In fact, γ-linolenic acid has been shown to reduce body fat (47) and facilitate fatty acid β-oxidation in the liver as judged by the increased activities of carnitine palmitoyl-transferase (24,47), acyl-CoA oxidase (24), and peroxisomal β-oxidation (47) in rats.

Exercise performance and increased GSH concentration. Except for the substrate-oriented explanation depicted in the previous paragraphs, the increased concentration of GSH may also explain to some extent the increased performance detected after spirulina supplementation. Several studies provided convincing data to support the view that cysteine is generally the limiting amino acid for GSH synthesis in humans and in other animals (54).

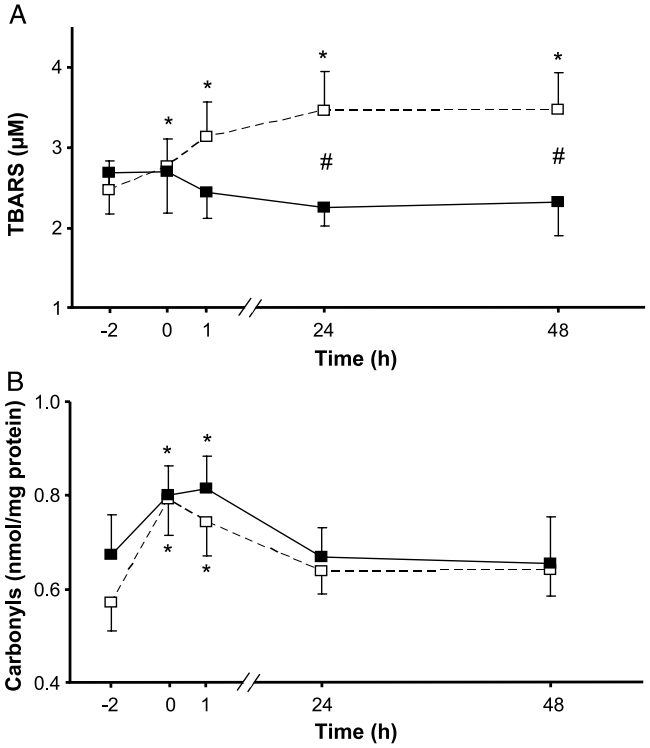

FIGURE 6—TBARS (*A*) and protein carbonyl (*B*) concentrations in the placebo (*open rectangles*) and spirulina exercise trials (*filled rectangles*; mean ± SEM). *Significantly different from the resting value in the same trial ($P < 0.05$). #Significantly different between placebo and spirulina trial at the same time point ($P < 0.05$).

Thus, increasing the supply of cysteine or its precursors (e.g., *N*-acetylcysteine) via oral or intravenous administration enhances GSH synthesis (28,44). Because cysteine can be generated from the catabolism of sulfur-containing methionine via the transsulfuration pathway, dietary methionine can replace cysteine to support GSH synthesis *in vivo* (54).

Spirulina contains 0.45 g of cysteine and 1.25 g of methionine per 100 g of dry spirulina (42). Given that subjects of the present study received 6 g spirulina per day, they received approximately 27 mg of cysteine and 75 mg methionine per day from spirulina. An analysis of the amino acid intake received by the subjects through their diet revealed that the subjects consumed approximately 3417 mg of cysteine and 6953 mg of methionine every day. This translates to a 0.79% increase in cysteine and 1.08% increase in methionine daily consumption solely from spirulina. It is possible that this small (but stable and dispersed throughout a day) administration of cysteine and methionine for the 4 wk of the supplementation period led to the increased concentration of GSH. Indeed, increased GSH concentration after spirulina supplementation has been reported in studies of the kidney (21,23), liver (21,38), lung (52), heart (48,52), and blood of rats (48).

Another potential mechanism that may have led to the increased levels of GSH after spirulina supplementation is the increased content of vitamins C and E in spirulina (54). In fact, vitamin C, vitamin E, and GSH undergo redox cycling *in vivo*, and there seeMSS to be a significant interrelationship among the three molecules in this cycling (54). Supporting this fact, several studies have indicated increased levels of GSH after supplementation with vitamins C and E (54).

GSH levels seem to be important in controlling the levels of RONS and muscle function (14). *N*-Acetylcysteine, a drug that supports GSH synthesis, has been consistently shown to delay muscle and whole-body fatigue. In humans, *N*-acetylcysteine administration improved performance of limb muscles (41) and diaphragm (51) during increased contractile activity protocols and extended time to failure during whole-body exercise (27,28). Overall, the role that RONS play in fatigue is still unclear (14), and consequently, the potential mechanisMSS through which the increased levels of GSH may have affected whole-body endurance in the present study are difficult to be predicted.

Effect of spirulina supplementation on redox status at rest. The only difference found in the present study regarding the redox status at rest was the higher concentration of GSH detected in spirulina-supplemented individuals. Despite a fair number of studies conducted in animals (e.g., [21,23,38,48,52]), we found only two studies that addressed the effects of spirulina supplementation on

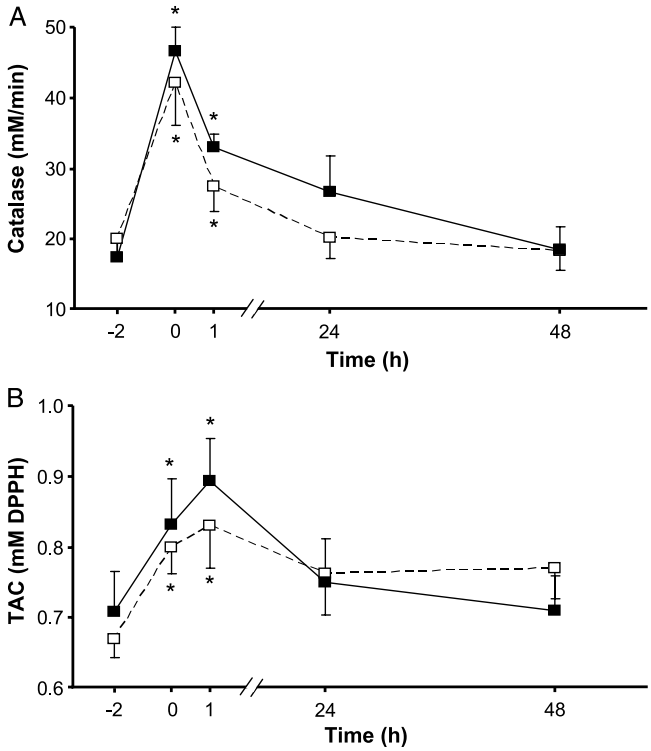

FIGURE 7—Catalase activity (*A*) and total antioxidant capacity (*B*) in the placebo (*open rectangles*) and spirulina exercise trials (*filled rectangles*; mean ± SEM). *Significantly different from the resting value in the same trial ($P < 0.05$).

redox status in humans (35,43). The two studies measured several indices of redox status in blood and reported contradictory results. For example, Park et al. (35) reported decreased levels of lipid peroxidation, whereas Shyam et al. (43) reported no change in lipid peroxidation after spirulina supplementation.

Effect of spirulina supplementation on redox status after exercise. TBARS was the only biochemical variable that a significant group × time interaction was detected, with TBARS levels increasing after exercise after placebo but not after spirulina supplementation. The main probable mechanism through which exercise increased lipid peroxidation after its cessation is the increased susceptibility to peroxidation of unsaturated fatty acids (16) because exercise markedly increases the concentration and unsaturation degree of nonesterified fatty acids in blood (32). The higher levels of GSH can partially explain the absence of an increase in lipid peroxidation after exercise in the spirulina-supplemented individuals. GSH can effectively scavenge several RONS that can cause lipid peroxidation (e.g., hydroxyl radical, lipid peroxyl radical, peroxynitrite, and hydrogen peroxide) directly and indirectly through enzymatic reactions (54). In addition, GSH is a substrate for glutathione peroxidase, which catalyzes the reduction of peroxides, such as hydrogen peroxide and lipid hydroperoxides (54). Another potential mechanism through which spirulina decreased lipid peroxidation might be the increased content of γ-linolenic acid in spirulina (33). Indeed, it has been found that an increased ratio of γ-linolenic acid to arachidonic acid is capable of attenuating the biosynthesis of arachidonic acid metabolites (i.e., prostaglandins, leukotrienes, and platelet-activating factor) and exerts an anti-inflammatory effect (9,19). Decreased inflammation via this route might have decreased the production of superoxide, hydrogen peroxide, and hypochlorous acid by the activated neutrophils (10) leading to less lipid peroxidation after spirulina supplementation.

Regarding the remaining indices of redox status (protein carbonyls, catalase, and TAC), all increased immediately and 1 h after exercise indicating oxidative stress. All redox status indices returned to their preexercise values at 24 h. Studies that have investigated the effects of aerobic exercise on serum protein carbonyls generally have reported increases similar to ours lasting up to 6 h of recovery (8,29).

Evidence addressing the efficacy of antioxidant supplementation to decrease oxidative stress remains ambiguous. For example, it has been shown that supplementation for 4 wk with vitamin E prevented the increase of lipid peroxidation after exercise (46). In addition, supplementation for 2 wk with vitamins C and E attenuated the rise in protein oxidation after exercise (8). On the contrary, supplementation for 6 wk with vitamin C, vitamin E, and β-carotene did not prevent the exercise-induced increase of lipid peroxidation (20). Moreover, supplementation for 5 wk with artichoke extract did not attenuate oxidative damage to erythrocytes after exercise (45). These differences in results may be related, in part, to the different concentration of the antioxidants and the combination of ingredients.

Mobilization of tissue antioxidant stores into plasma, such as uric acid (13), is probably one mechanism responsible for the marked increase (and not decrease, as might be expected intuitively) of TAC after exercise. This is a widely accepted phenomenon that helps maintain or even increase serum antioxidant status in times of need (39). Increased catalase activity after exercise also could have contributed to the increased TAC. Nevertheless, this increase in the antioxidant capacity of serum did not prove efficient at inhibiting the increase in lipid and protein oxidation in the blood. Most studies agree that exercise increases TAC for some hours after exercise (3,53). Perhaps the increased TAC could mean that the plasma gets enriched with antioxidant molecules that need to be transported into tissues where they can provide protection.

CONCLUSIONS

Many positive claiMSS for spirulina are based on research done on individual nutrients that spirulina contains, such as various antioxidants, rather than on direct research using spirulina. This is one of the few studies where humans were supplemented with spirulina. We report for the first time that supplementation of spirulina for 4 wk increased exercise performance, possibly through an increase in fat oxidation rate, and increased GSH levels. The reasons behind the enhanced performance and increased fat oxidation after spirulina supplementation are poorly understood, and more research is needed to elucidate this. Particularly, the effect of spirulina on mitochondrial function and β oxidation in conjunction with inflammation and oxidative stress requires further investigation.

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