frontiers | Frontiers in Nutrition

TYPE Systematic Review
PUBLISHED 14 December 2022
DOI 10.3389/fnut.2022.1048258

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

RECEIVED 19 September 2022
ACCEPTED 21 November 2022
PUBLISHED 14 December 2022

CITATION
Calella P, Cerullo G, Di Dio M, Liguori F, Di Onofrio V, Gallè F and Liguori G (2022) Antioxidant, anti-inflammatory and immunomodulatory effects of spirulina in exercise and sport: A systematic review. *Front. Nutr.* 9:1048258. doi: 10.3389/fnut.2022.1048258

Antioxidant, anti-inflammatory and immunomodulatory effects of spirulina in exercise and sport: A systematic review

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Arthrospira platensis, also known as spirulina, is currently one of the most well-known algae supplements, mainly due to its high content of bioactive compounds that may promote human health. Some authors have hypothesized that spirulina consumption could protect subjects from exercise-induced oxidative stress, accelerate recovery by reducing muscle damage, and stimulate the immune system. Based on this, the main goal of this review was to critically analyze the effects of spirulina on oxidative stress, immune system, inflammation and performance in athletes and people undergoing exercise interventions. Of the 981 articles found, 428 studies were considered eligible and 13 met the established criteria and were included in this systematic review. Most recently spirulina supplementation has demonstrated ergogenic potential during submaximal exercise, increasing oxygen uptake and improving exercise tolerance. Nevertheless, spirulina supplementation does not seem to enhance physical performance in power athletes. Considering that data supporting benefits to the immune system from spirulina supplementation is still lacking, overall evidence regarding the benefit of spirulina supplementation in healthy people engaged in physical exercise is scarce and not consistent. Currently, spirulina supplementation might be considered in athletes who do not meet the recommended dietary intake of antioxidants. Further high-quality research is needed to evaluate the effects of spirulina consumption on performance, the immune system and recovery in athletes and active people.

Systematic review registration: [https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=262896], identifier [CRD42021262896].

KEYWORDS

spirulina, antioxidant, immunomodulatory, anti-inflammatory, athletes

Introduction

Arthrospira platensis, commonly named spirulina (SP), is a type of blue-green algae, which is a member of the phylum Cyanobacteria (1). It contains proteins (phycocyanin), B-group vitamins, natural colors (chlorophyll and carotenoids), and important fatty acids. Since the 16th century, SP has been generally accepted as a food and dietary supplement (2). It is used as a nutraceutical food supplement due to its high protein (up to 65% dry weight) and bioactive compound content including many phytonutrients, such as β-carotene, echinenone, zeaxanthin, 3-hydroxyechinenone, c-phycocyanin, which all have strong antioxidant activity (3). It has been reported that SP improves macrophage activity, natural-killer (NK) cells proliferation, activation of T-cells, up-regulating key cells and organs of the immune system enhancing their ability to counteract the action of infectious agents and the activity of environmental toxins. The potential therapeutic applications of SP, based on its immunomodulatory and anti-inflammatory activities, have already been reviewed by several authors (4–7).

Beyond the clinical implications, some authors have hypothesized that SP supplementation could also be advantageous for healthy, active individuals, especially athletes. For example, SP could modulate markers of exercise-induced lipid peroxidation, such as plasma thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA) and protein carbonyls (PC), as well as improving the activity of redox enzymes such as catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD), suggesting a role in the management of oxidative stress (8, 9). People involved in high-intensity physical training increase the production of reactive oxygen species (ROS) and need to follow a well-balanced diet that satisfies their requirements for energy, macro- and micronutrients, in order to maintain an optimal redox state and avoid potential immune dysfunction (10). However, bearing in mind that ROS production during exercise has a pivotal role in long-term training adaptation (11–13), excessive oxidative damage could have a negative impact on the immune system (14), recovery (15), ability to perform and general health (15, 16). According to anecdotal evidence, the Chinese and Cuban Olympic teams have been taking SP daily for many years and have performed better (17). Early *in vitro* reports demonstrating the high radical scavenging activity of the algae (18), probably involving the activation of the NRF2 signaling pathway (6, 19) and the prevention of lipid peroxidation (18), have sparked a lot of interest in how the antioxidant effects of SP supplementation may support exercise performance. Other authors have shown that SP could accelerate recovery by reducing creatine kinase (CK), lactate dehydrogenase (LDH) and C-reactive protein (CRP) (9, 20); strengthen the immune system (21–23); and improve performance in various situations (9, 24).

The potential effects of SP supplementation on performance, immunity, exercise-induced muscle damage, and recovery in athletes have not been considered in the recommendations published so far (25–29). Only Braakhaius et al. and Gurney et al. discussed the impact of SP supplementation on athletic performance, focusing on its antioxidant and ergogenic properties (30, 31).

To our knowledge, the effects of SP supplementation in athletes and healthy people engaged in exercise have not been systematically reviewed; thus, the main aim of this review is to critically appraise the literature on the exercise-related effects of SP supplementation on oxidative stress, immune system, inflammation and performance.

Materials and methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed for this systematic review (32) also taking into account the updating in the motor-sports field and the 27 points of the PERSiST guidelines (33). The research protocol has been registered in the PROSPERO database (reg. n. CRD42021262896) with limitations.

Eligibility criteria

For this review studies have been included which investigated the effects of SP supplementation in humans of any age, who regularly played sports at any level or underwent exercise interventions, without diagnosed pathologies or disorders. Only randomized controlled studies reporting the dose of SP administered were included in the analysis.

Outcomes

How SP affects the modulation of oxidative stress, exertional inflammation, the regulation of the immune system and whether it has effects on performance and fatigue.

Literature search and selection of studies

The search for literature was carried out until 23 July 2022 on the following electronic databases: (1) MEDLINE (Pubmed); (2) Scopus; (3) SPORTDiscus (EBSCO) and (4) Google Scholar. The main search string included the following words: (Spirulina OR Arthrospire OR Blue-Green Algae) AND (antioxidant OR antioxidants OR immunomodulatory OR immunomodulation OR immune system OR anti-inflammatory activities OR inflammation) AND (athlete OR athletes OR sport OR exercise OR physical exercise OR physical activity

OR training). Moreover, the reference lists of included studies and pertinent reviews were also analyzed to identify further articles. The search was not restricted by date or publication status. Articles in English, Spanish, Italian and French were considered.

Data collection

The results of the electronic search were evaluated independently by two reviewers (PC and GC). Duplicate studies were eliminated, and potentially eligible studies were identified and chosen based on title and abstract. Two reviewers (PC and GC) carefully screened the full text of the potentially eligible studies, and those that met the selection criteria were considered for the analysis. A third reviewer's opinion was used to settle disagreements (VDO).

The following information was identified for each study by four reviewers (PC, GC, MD, and FL): author, year and country of publication, main purpose of the study, sample size, type of study design, participant characteristics (number, mean age, sex), supplementation dose, length of intervention and main results (**Table 1**).

Quality assessment

Three authors (GC, PC, and MD) separately assessed the risk of bias of each included study using the revised Cochrane Risk-of-Bias tool for randomized trials (RoB2) (34). Disagreements were resolved by consultation with a third reviewer (FG) (34, 35).

Results

Study selection and characteristics

As shown in **Figure 1**, the primary search identified 981 relevant articles, 428 of which were assessed after duplicates had been removed and the titles and abstracts screened. According to the search topic and the inclusion criteria, 13 studies were included in the present systematic review (8, 9, 20, 22, 24, 36–43) (**Table 1**).

In total, 267 participants were analyzed. Only 3 studies involved female participants (9, 24, 43). The majority of the studies dealt with adult participants with a mean age of between 20 and 30 years; only two studies had participants with a mean age of 40 ± 8 (42) and 51 ± 3 years (43).

Spirulina (SP) supplements were mainly used at the dosages of 3 to 6 g/day, one study (9) used SP at higher dosages (7.5 g/day in college students) while three studies used less than 3 g/day of SP supplements (22, 24, 43). Most of the studies used SP supplements at the dosages of 1 to 6 g/day (ranging from 500 mg/d to 7.5 g/d). The duration of intervention ranged from 3 to 8 weeks in the majority of the studies (8, 9, 20, 22, 24, 37, 39, 40, 43), one study (43) was longer (12 weeks) and three studies lasted between 4 and 21 days (36, 41, 42).

Five studies involved athletes (20, 22, 37–39), five included trained subjects (8, 24, 36, 40, 42) and three were carried out on untrained people undergoing exercise (9, 41, 43).

As for the quality, three studies showed a low risk of bias, eight studies showed a high risk of bias and two studies had some concerns (**Figure 2**).

Studies in athletes

Two studies, conducted by the same laboratory, reported the effects of SP supplementation in elite rugby players (20, 37). The first reported no significant difference between groups for squat jumps, countermovement jumps, and 10- and 30-m sprints after SP supplementation (37). The second demonstrated that SP supplementation prevents exercise-induced lipid peroxidation (F2-Isop), inflammation (CRP), and skeletal muscle damage (CK) and also accelerates the recovery of some of these markers immediately after and 24h after exhaustive exercise. However, other markers of redox state (SOD, GPx, oxidized low-density lipoprotein and glutathione/glutathione disulfide ratio or GSH/GSSG), inflammation (myeloperoxidase), and muscle damage (LDH), did not differ significantly between groups (20).

Two studies obtained opposing results, regarding protection against exercise-induced lipid peroxidation after SP supplementation (38, 39) in endurance exercise. In fact, SP did not interfere in the magnitude of oxidative stress (MDA and SOD) nor in muscle damage (CK and LDH) in regional-level cyclists, subjected to high volume and intensity of training (six sessions/week, 2-6h per session) (39).

However, the supplementation of SP resulted in a significant decrease in the level of MDA in young (aged 15–21) Indian male athletes (38). The same study demonstrated that supplementation of SP enhanced the levels of serum β-carotene, serum α-tocopherol, and plasma ascorbic acid in a similar way to a commercial antioxidant supplement (Selace Forte©, which mainly contained: Vit C-500 mg, Vitamin E-400 mg, Carotenoids-12.5 mg) (38).

Only one study, carried out on rowers of the Polish Rowing Team, showed that SP could influence the immune system (22). According to Juszkiewicz et al., SP may protect athletes against a deficit in immune function related to strenuous exercise by reducing the post-exercise increase in cells and regulatory T-cell count (22). In more detail, participants from the placebo group had a significant post-recovery increase in Treg/(NK cells + Tδγ + cytotoxic lymphocytes) ratio, which was absent in the SP group. Nonetheless, no ergogenic effect was observed in a 2000-m rowing ergometer test (22).

TABLE 1 Sample and intervention characteristics of the studies.

Author, year, country	Study design	Sample characteristics (spirulina)	Sample characteristics (placebo and control group)	Daily dose, intervention length and placebo	Exercise protocol	Outcome	Main results
Chaouachi et al. (37), France	DB, parallel	$n = 11$, 25.8 ± 3.4 years, elite male Rugby Union players	$n = 11$, 26.3 ± 4.4 years, elite male Rugby Union players	5.7g, 7 weeks, 70.3% egg proteins and 29.7% carbohydrates	Concentric (three maximal knee extensions and flexions, at 60 and 240° s^{-1}) and eccentric (three maximal contractions at 30° s^{-1}) measurements with 2min rest between series, vertical jumps, running speed using a stationary 10-m and 30-m sprint test and Yoyo IRT-1.	Anthropometric measurements (BM, FM) and physical performance (isokinetic leg strength and power, VJ, speed and Yoyo IRT-1)	No significant differences between groups
Chaouachi et al. (20), France	DB, parallel	$n = 9$, 25.2 ± 2.7 years, elite male Rugby Union players	$n = 8$, 25.9 ± 3.3 years, elite male Rugby Union players	5.7g, 7 weeks, 70% egg proteins and 30% carbohydrates	Repeated high−intensity exercise bouts consisting of repeated 40 m (2 × 20 m) runs between markers set 20m apart, at a progressively increased speed.	Redox status (GSH, GSSG, SOD, GPx, ox-LDL and F2-Isop), inflammation (CRP and MPO) and muscle damage (CK and LDH)	↓CRP ↓CK ↓F2-Isop
Franca et al. (39), Brazil	DB, parallel	$n = 11$, 27.8 ± 3.5 years, male cyclists	$n = 7$, 34.3 ± 2.3 years, male cyclists	7.5g, 4 weeks, corn starch	High volume and intensity training regimes (six sessions/week, 2-6h per session)	Muscle damage (CK and LDH) and redox status (MDA and SOD)	No significant differences between groups
Gurney and Spendiff (41), England	DB, cross-over	$n = 11$, 21.1 ± 1.0 years, males untrained in arm cycling	$n = 11$, 21.1 ± 1.0 years, males untrained in arm cycling	6g, 7 days, soy protein	30-min bout of submaximal upper body cycling exercise at 55% $\dot{V}O_{2max}$, followed by an incremental test to fatigue using Arm Crank Ergometry.	Blood Hb, respiratory variables (RER, HR, Oxygen Uptake) and Time to fatigue	↑Hb ↑Oxygen Uptake ↓HR ↔ RER ↔Time to fatigue
Gurney et al. (42), England	DB, cross-over	$n = 15$, 40 ± 8 years, male cyclists	$n = 15$, 40 ± 8 years, male cyclists	6g, 21 days, microcrystalline cellulose	1-h submaximal endurance test at 55% external power output max and a 16.1km time trial (day 1), followed by a lactate threshold test and repeated sprint performance tests (day 2).	Blood parameters (Hb, glucose, lactate) respiratory variables (RER, HR, Oxygen Uptake) and exercise performance (time and power output)	↑Hb ↓HR ↑Power output (peak and average) ↔Oxygen Uptake ↔Time trial
Johnson et al. (40), USA	DB, parallel	$n = 9$, 20-43 years, active men	$n = 8$, 20-43 years, active men	3g, 8 weeks, gelatin capsules	30-min bout on a cross training, elliptical machine.	Physical (kcal in 30 min) and mental fatigue (15 min computer version of the Uchida–Kraepelin test).	↑ Physical fatigue (after 1 week) ↔ Physical fatigue (after 8 weeks) ↓Mental fatigue (both 1 and 8 weeks)
Juszkiewicz et al. (22), Poland	DB, parallel	$n = 10$, 20.4 ± 0.84 years, male Polish rowing team	$n = 9$, 20.0 ± 0.71 years, male Polish rowing team	1.5g, 6 weeks, calcium gluconate	2000-m time trial test on a rowing ergometer	Immune system (Tregs, CTLs, NK cells and Tδγ cells) and exercise performance (2000m time trial test)	↓Treg/CTL ↑Treg/(NK + Tδγ + CTL) ratio

(Continued)

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TABLE 1 (Continued)

Author, year, country	Study design	Sample characteristics (spirulina)	Sample charac teristics (placebo and control group)	Daily dose, intervention length and placebo	Exercise protocol	Outcome	Main results
Kalafati et al. (8), Greece	DB, cross-over	n = 9, 23.3 ± 1.7 years, male recreational runners	n = 9, 23.3 ± 1.7 years, male recreational runners	6g, 4 weeks, egg proteins	2h treadmill at 70%VO_{2max} + 95% VO_{2max} to exhaustion	Exercise performance (time to fatigue), exercise metabolism, redox status (GSH, GSSG, GSH/GSSG, TBARS, PC, CAT, TAC) and muscle damage (CK). (0, 1h, 24h, 48h post-exercise)	↓Carbohydrate oxidation rate ↑Fat oxidation rate ↑GSH (at rest and 24 h post-exercise) ↑Time to fatigue ↔CK
Kalpana et al. (38), India	DB, parallel	n = 30, 15-21 years, Indian male athletes	n = 30, 15-21 years control group (no supplementation), Indian male athletes n = 30, 15-21 years, group supplemented with commercially antioxidant (Selace Forte©, Indian male athletes	3g, 60 days, no placebo	Regular training mantained	Redox status (serum tocopherol, ascorbic acid, β-carotene, MDA).	↓MDA ↑Serum tocopherol ↑Ascorbic acid ↑β-carotene
Lu et al. (9), Taiwan	DB, parallel	n = 8, 20.00 ± 0.69 years, untrained students (3 male + 5 female)	n = 8, 21.43 ± 1.02 years, untrained students (3 male + 5 female)	7.5g, 3 weeks, soy protein	Exhaustive exercise (treadmill exercise following the Bruce incremental protocol)	Muscle damage (CK and LDH), redox status (MDA, GPx and SOD), blood lactate and time to exhaustion.	↑SOD ↓MDA ↑GPx ↑Time to exhaustion ↓LDH ↔CK
Pappas et al. (36), Greece	Parallel	n = 12, 22.5 ± 4.3 years, recreationally trained males	n = 12, 21.2 ± 2.2 years, recreationally trained males	6g, 4 days, wheat flour	Maximal eccentric voluntary contractions (5 × 15 with 2min rest) at an angular velocity of 60/s performed on an isokinetic dynamometer (knee range,0° full extension to 90° flexion)	Redox status (TAC and PC), muscle performance (EPT) and muscle damage (DOMS) (0,24h,48h,96h post exercise)	No significant differences between groups
Sadeghi et al. (43), Iran	Parallel	n = 12 (SP), 50.3 ± 2.9 years, inactive women n = 10 (SP + training), 51.5 ± 3.4 years, inactive women	n = 10 (control group, only training), 51.7 ± 2.3 years, inactive women	500mg, 12 weeks, no placebo	Regular training: 3d/week resistance training	Homocystein, anthropometry variables (waist to hip ratio and BMI)	↓Homocystein in SP + training compared to only SP or training
Sandhu et al. (24), India	DB, parallel	n = 10, 25.2 ± 3.5 years, untrained n = 10, 24.4 ± 3.4 years, trained n tot = 40 (22 men, 18 woman)	n = 10, 25.2 ± 3.5 years, untrained n = 10, 24.4 ± 3.4 years, trained n tot = 40 (22 men, 18 woman)	2g, 8 weeks, flour	2 isometric maximal voluntary contractions of the dominant quadriceps at 60° knee flexion with 10s and 60s hold with a rest period of 2 min.	Isometric strength (peak force and average force) and fatigue index.	↑Peak force ↑Average force ↔Fatigue index

BM, body mass; BMI, body mass index; CK, creatine kinase; CAT, catalase; CRP, C-reactive protein; CTL, cytotoxic lymphocytes; DB, double blind; DOMS, delayed onset muscle soreness; EPT, eccentric peak torque; F2-Isop, F2 α- isoprostanes; FM, fat mass; GPx, glutathione peroxidase; GSH, glutathione; GSSG, glutathione disulfide; h, hours; Hb, hemoglobin; HR, heart rate; IL-6, interleukin-6; IRT-1, intermittent Recovery Test Level 1; LDH, lactate dehydrogenase; MDA, malondialdehyde; min, minutes; MPO, myeloperoxidase; n, sample size; NK, natural killer; ox-LDL, oxidized low-density lipoprotein; PC, protein carbonyls; RER, respiratory exchange ratio; SOD, superoxide dismutase; TAC, total antioxidant capacity; TBARS, plasma thiobarbituric acid reactive substances; VJ, vertical jump; VO_{2max}, maximal oxygen uptake.

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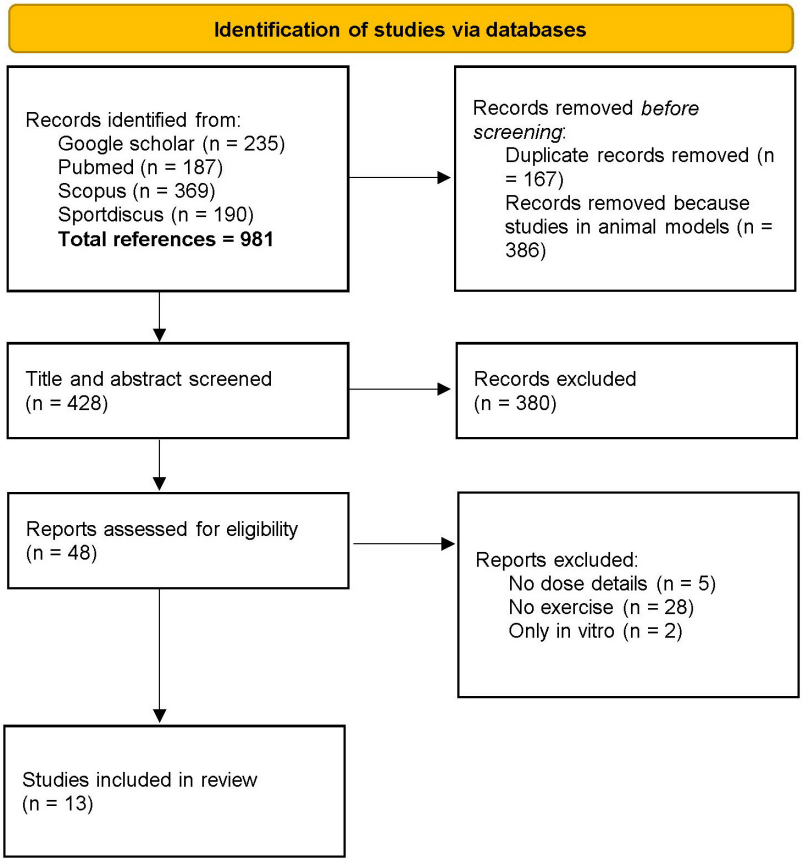

FIGURE 1
Prisma flow diagram of the selection of the articles included.

Studies in trained subjects

Two studies explored the effect of SP supplementation on muscle strength, reporting different results (24, 36). According to Sandhu et al., SP was effective in increasing peak isometric muscle strength, average force and reducing fatigue similarly in trained and untrained volunteers (24). On the other hand, in recreationally trained males, SP did not confer beneficial effects on peak torque (EPT), redox status [Total antioxidant capacity (TAC) and PC] or delayed onset muscle soreness (DOMS) immediately after exercise, as well as at 24, 48, 72, and 96 h post exercise (eccentric contractions consisting of 5 sets and 15 maximum reps per set) (36). Some authors observed an improved exercise tolerance [time to fatigue at 95% of maximal oxygen uptake (VO_2max) after a 2-h run at 70%–75% of VO_2max] in recreational runners after SP supplementation together with an attenuated exercise-induced increase in lipid peroxidation (TBARS) (8). However, they failed to show any significant effect on muscle damage (CK) after the exercise protocol (8).

In trained cyclists, SP significantly increased hemoglobin (Hb) and reduced heart rate (HR) during submaximal exercise (42). Although results did not show improvement in exercise performance (16.1km time trial after 1-h submaximal endurance test), SP supplementation significantly increased power output during repeated sprint performance tests (42).

One study reported an improvement in indices of mental fatigue in men 4 h after the first supplementation as well as 8 weeks later and also a statistically significant increase in exercise output (Kcals consumed in 30 min exercise on a cross trainer machine) after SP supplementation (40).

Studies in untrained people

Sadeghi et al. found that improvement in homocysteine levels were significantly greater in inactive females after SP combined training (43) compared with only training (43). The training protocol included three sessions a week of supervised training consisting of resistance exercise plus steady-state exercise performed at an intensity of 60–70% maximal heart rate for 30 min.

One study examined the effects of SP on redox status and muscle damage in college students by comparing the results of the Bruce incremental treadmill exercise before

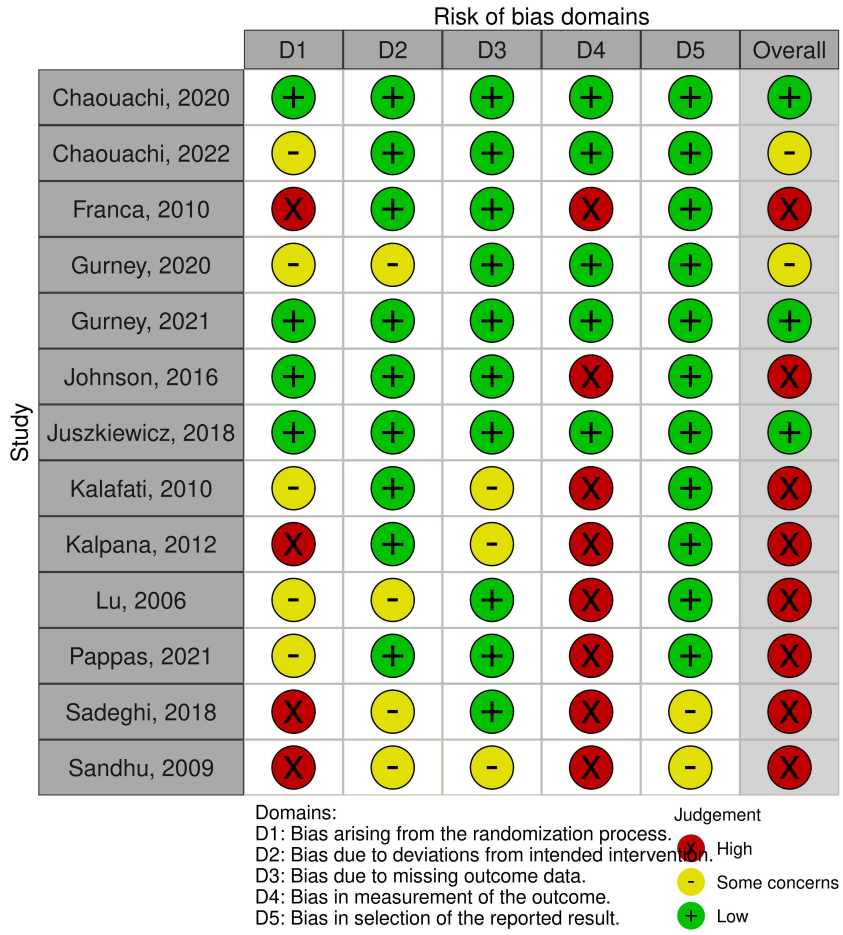

FIGURE 2
Methodological quality of the studies using the tool RoB 2.0.

and after SP treatment (9). The results showed that plasma concentrations of MDA and LDH were significantly lower after supplementation with SP, while the activity of SOD and GPx significantly improved. Furthermore, time to exhaustion (TE) was significantly extended, leading the authors to speculate that ingestion of SP may confer protection against muscle damage related to exercise (9).

Spirulina (SP) supplementation significantly enhanced oxygen uptake and Hb during arm cycling submaximal exercise (30-min submaximal exercise bouts, corresponding to 55% of $\dot{V}O_2$max, followed by an incremental test to fatigue) in males untrained in arm cycling (41). However, time taken to fatigue was not different.

Discussion

The studies analyzed by this review have been written in the last 20 years and evaluate the exercise-related effects of SP supplementation. Antioxidant, anti-inflammatory, immunomodulatory and ergogenic effects of SP have been observed. Research findings on the use of SP in sports, with a focus on ergogenic effects and influence on recovery and the immune system, are summarized in **Table 2**.

Antioxidant effects

Evidence on the antioxidant effects of SP is mixed. According to Kalafati et al., SP supplementation improved GSH concentration and attenuated the exercise-induced increase in lipid peroxidation, thereby inducing a significant improvement in exercise performance (8). However, authors reported a lower daily intake level of vitamin A and selenium in the SP group. In concert with vitamin E and GPx, selenium helps minimize the generation of harmful free radicals, especially during endurance exercise, hence benefits in the SP group may have been influenced by differences in daily antioxidant dietary intake between groups (placebo and SP) (8). Other findings suggested that SP supplementation did not confer beneficial effects on

redox status, performance, or muscle damage following a muscle damaging protocol based on eccentric exercise (36). However, it should be considered that a single bout of exercise based on only eccentric contraction does not represent real life conditions.

Two studies agreed that SP supplementation reduces oxidative damage by significantly lowering MDA concentrations and raising blood SOD activity (9, 38). The supplementation of SP also enhanced the levels of antioxidants in the blood with greater benefits on the redox state in endurance than in mixed sports (38). In this case, it is important to note that all participants' intake of β-carotene, iron, and zinc was lower than the recommended daily allowance (38). Indeed, similar benefits on SOD and MDA were not observed in cyclists with adequate nutritional status (39) or elite rugby players (20).

Overall, there are several things to point out. First, many studies used TBARS, TAC or MDA to assess lipid peroxidation (8, 9, 36, 38, 39). Lipid peroxidation is still a valuable marker of exercise-induced macromolecule damage (44), however, considering that most MDA is generated by the assay itself (i.e., artificial lipid peroxidation), two or more indices are usually required to confirm lipid damage (45). Furthermore, the TBARS and TAC assays are no longer recommended for assessing lipid peroxidation (46) and should be discontinued as strongly advised by many experts (31, 46). In addition, elevated levels of GSH/GSSG, which are often used as a marker of oxidative stress after exercise, have also been reported due to methodological errors (47).

Equivocal results could potentially be related to inter-individual baseline variability in antioxidant compounds prior to beginning supplementation. In this sense, results should be carefully interpreted based on participant characteristics, protocol used and control group or placebo.

Lastly, supplementation for redox balance management during and after exercise is controversial since it may interfere with numerous ROS-mediated mechanisms that influence training adaption, including mitochondrial biogenesis and hypertrophy (11, 48–52). Accumulating evidence suggests that exercise-induced reactive species are required for the activation of signals regulated by redox-sensitive transcription factors (PCG-1, HIF-1α, NF-κB, and NFE2L2) (13, 50, 53). There has been no research into how SP may interfere with ROS-mediated adaptations such as strength, hypertrophy, or endurance, and further investigation is urgently needed in this sense.

Although it is clear that SP has a wide range of components capable of free radical scavenging *in vitro*, there is still a significant knowledge gap regarding the antioxidant benefits of SP supplementation in athletes or people engaged in exercise in general (31).

Effects on inflammation

Beyond redox state, four studies explored the anti-inflammatory action of SP (8, 9, 20, 39). Of these studies, two involved athletes (20, 39). Indeed, an inflammatory response (54) associated with muscle damage during and after exercise exists, which leads to increases in inflammatory markers like CRP (55–57) and intracellular proteins like CK (55), and the release of cytokines like tumor necrosis factor (TNF-α) and IL-6 in order to repair damaged tissue (58). This inflammatory status frequently causes muscle pain and functional decline (55), impairing overall performance (51, 59).

Studies in animal models have reported an anti-inflammatory effect of SP (60, 61) comparable to that of diclofenac sodium (60). In humans, results from a recent meta-analysis of controlled clinical trials demonstrated that SP supplementation resulted in a significant reduction of IL-6 concentrations when the baseline body mass index (BMI) of participants was lower than 25 kg/m^2 (62). Almost none of the studies included in our work that evaluated the effects of SP on muscle damage showed any benefit on CK (8, 9, 39) and DOMS (36), suggesting no implication in

TABLE 2 Summary of research findings on the use of Spirulina in sport.

Ergogenic effect		A supplementation of 6–7.5 g/day could be considered in athletes engaged in a high volume of submaximal endurance training (e.g., cyclists or runners) in order to improve redox status, fatigue tolerance and hemoglobin level (8, 9, 41, 42). SP supplementation does not seem to improve physical performance in power athletes (22, 37).
Recovery		The majority of studies showed no benefit on CK (8, 9, 39) or DOMS (36) suggesting no implication in muscle recovery. 5.7g/day of SP seems to accelerate recovery after training/competitions in elite rugby players (20). SP supplementation may potentially prevent exercise-induced oxidative damage, inflammation, and muscle damage in elite athletes who do not achieve the recommended antioxidant dietary intake (20, 38).
Immune system		It is hypothesized that SP can protect athletes against immune dysfunction associated with heavy exercise. SP may play a role in the maintenance of lower Treg counts in tissues, preventing the immunosuppressive effects of these cells and restoring an immune balance (22). Currently, evidence supporting the benefit to the immune system through SP supplementation remains scarce.

CK, creatine kinase, DOMS, delayed onset muscle soreness, SP, spirulina.

muscle recovery. Only recently, SP supplementation has been shown to have a role in the prevention of exercise-induced inflammation and skeletal muscle damage by reducing CK, CRP and F2-Isop levels immediately and 24h after exercise in elite rugby players (20). Although these results seem to be favorable, the mechanisms behind the above-mentioned effects of SP related to exercise are still poorly understood. According to our systematic review, no studies have yet considered the effects of SP on TNF-α, IL-6, and CRP in healthy subjects and/or athletes, and more research should be conducted in this area.

Immunomodulatory properties

Innate and acquired immunity have both been reported to decline temporarily in the hours following strenuous exercise (by up to 70%) basically giving an "open window" for opportunistic infections (29). Effector lymphocytes, such as NK cells, T lymphocytes (helper and cytotoxic), TCR δγ-positive (Tδγ) cells and regulatory T cells (Tregs) play a crucial role in cell-mediated immune response (29). Some authors have documented SP's ability to affect the immune system in healthy individuals (23, 63). In 2009, Milasius et al. reported for the first time that SP supplementation had a positive effect on the quantitative indices of immune response in high performance sportsmen (21).

Exposure to a high stressor, such as maximal exercise, nonetheless results in an increase/decrease both in the number of cytotoxic cells and in Treg count. Based on the results of Juszkiewicz et al., SP supplementation did not exert a statistically significant effect on Treg count in members of the Polish Rowing Team (22). However, athletes in the placebo group showed a significant post-recovery increase in the Treg/(NK + Tδγ + cytotoxic) ratio, which was lacking in the supplemented group, leading the authors to speculate that SP supplementation may protect athletes from immunological deficiencies. Currently, the study published by Juszkiewicz and colleagues remains the only randomized study to have investigated the effects of SP in athletes (22).

There is little evidence to support the idea that athletes should suppress their immune systems, yet taking supplements to boost immune systems is still a controversial topic due to the multiple mechanisms that modulate immune response during and after exercise (29). According to a new paradigm for exercise immunology proposed by Walsh that considers "resistance" (the strength of the immune weaponry) and "tolerance" (the ability to endure microbes and dampen defense activity), it is not surprising that supplements designed to increase immune "resistance" have little or no efficacy (29). There is growing knowledge that episodes of upper respiratory symptoms (URS) usually cluster around intense periods of training or competition (64–66), especially during winter months (66, 67) leading to respiratory inflammation, characterized by a dysregulated anti-inflammatory response and oxidative stress (68). Although athletes generally consume a nutrient-dense diet rich in fruit and vegetables (69, 70), SP may improve tolerance, mitigating tissue damage during exercise or infection, and improve recovery (67). New studies should be designed to explore the potential tolerogenic properties of SP, focusing on the prevention or treatment of URS.

The efficacy of immunonutrition techniques should be investigated using multi-omics approaches. As pointed out by many authors, metabolomics, lipidomics, and proteomics allow the simultaneous evaluation of a large number of small-molecule metabolites, lipids, and proteins, providing a system-wide overview of the metabolic response to exercise and nutritional interventions (71–73). Future studies should contemplate the use of a human systems biology approach with multi-omics outcomes to better understand whether or not SP can aid athletes.

Nowadays, evidence supporting the benefit of SP supplementation on the immune system remains scarce and it is still too early to encourage its use for this purpose.

Ergogenic effect

Although antioxidant benefits are typically the main focus of SP supplementation, several authors have reported promising results of SP as an ergogenic aid, proposing multiple pathways that may be responsible for the observed improvements. For instance, there is growing evidence that SP can improve submaximal exercise and fatigue tolerance (8, 9, 40, 42). These benefits could be attributable to several factors. Firstly, an ergogenic effect may be due to the antioxidant effect of SP: the contraction-induced ROS generation is associated with oxidative damage and earlier onset of fatigue (11, 15). Unsurprisingly, improvements in exercise performance were often observed together with an enhanced redox state at rest after SP supplementation (8, 9). Some evidence in rats has shown that SP can have vasodilatory effects. For instance, SP could enhance circulatory nitrate/nitrite, increasing nitric oxide availability and the expression of endothelial nitric oxide synthase (74, 75). However, despite enhanced nitrate intake potentially representing a good strategy for improving performance (76–79), increases in nitrate/nitrite following SP supplementation in humans have not yet been reported.

Secondly, the high iron concentration of SP may contribute to its ergogenic effects. It is commonly recognized that adequate iron stores are critical for regular Hb synthesis and that Hb is essential for the transport of oxygen from the lungs to the muscles (80). Due to the lack of phytates and oxalates, SP iron is easily absorbed by the body. This has led to some positive

hemopoietic trends in athletes and healthy, active men without a known iron deficiency (21, 41). In particular, some authors have demonstrated that SP supplementation may enhance oxidative capacity (8) and significantly increase Hb during submaximal exercise (41, 42). To the best of our knowledge, SP has exclusively been investigated in male athletes and, considering the high prevalence of anemia in female athletes, future studies should involve female participants, as recently highlighted by Gurney and Spendiff (31).

Finally, SP supplementation could improve performance by alleviating mental fatigue, as shown by Johnson et al. (40). However, the evaluation of fatigue remains a very complex topic. As recently proposed, fatigue should be defined in terms of fatigability (perceived and performance fatigability) and should not be combined with any adjective, such as mental fatigue, muscle fatigue or physical fatigue (81, 82). Further studies designed with protocols that allow the assessment of performance fatigability during exercise are encouraged (83–85), in order to understand the anti-fatigue implications of SP supplementation.

Considering the effects of SP on muscle strength and power performance, all studies but one (24) failed to show significant improvements (22, 36, 37). SP supplementation was effective in increasing peak force and average force of the quadriceps in trained and untrained individuals. Nevertheless, the psychological and motivational components of the subject during the testing of maximal voluntary isometric contraction might have affected the outcome of the study, as reported by the authors in the limitations of their study (24). In elite male rugby players during the competitive phase, SP supplementation did not improve maximal strength and power (37). Also, in members of the Polish rowing team, SP supplementation did not show ergogenic effects (22). Differences in doses (1.5g–6g), intervention lengths (4 days–8 weeks), training status (from untrained to elite athletes) and protocols, make it difficult to compare findings from studies evaluating the impact of SP on muscle strength and power performance.

Strengths and limitations

This systematic review included all published research that has looked at the effects of SP supplementation considering its antioxidant, immunomodulatory, and anti-inflammatory activities, underlying biochemical mechanisms and practical implications. The reviews on this topic often focused on a single mechanism of action of SP cataloging its effects only at the level of some biological compounds or on some clinical aspects. With this systematic review we wanted to focus on the proven main properties of this compound analyzing the known effects of SP on immune response and inflammatory processes in athletes, and on the consequences that it may have in terms of athletic performance and recovery.

However, when interpreting our findings, several limitations should be acknowledged. First, given the specificity of the target – athletes and people doing exercise, we decided to analyze the literature regarding the anti-oxidant, immunomodulatory and anti-inflammatory effects of SP in these groups separately from the other records found following the registered protocol. As a consequence, this systematic review included 13 studies with only 267 study participants, and the majority of the trials involved small groups of subjects randomly allocated to parallel groups. Furthermore, in the included articles, SP was given in varied quantities or forms for a variable duration of intervention and even the characteristics of study participants varied widely. Moreover, the different exercise protocols and participants' training status make it difficult to compare results. Finally, it is important to underline that the quality of these studies was generally poor; indeed, only three studies showed a low risk of bias. All these aspects hinder the validity of our findings and do not allow to express definite evidence on the use of SP in exercise and sport. New, higher quality research is needed to understand under what conditions and in what type of individuals SP supplementation may be recommended.

Conclusion

Considering the effects of SP on exercise-related oxidative stress, equivocal results could potentially be related to inter-individual baseline variability in antioxidant compounds, participants' characteristics (e.g., age and sex), and training status. Based on this, there is still a significant knowledge gap regarding the antioxidant benefits of SP supplementation in athletes or people engaged in exercise in general.

Emerging evidence suggests that SP could be useful during submaximal endurance exercise, increasing oxygen uptake and improving exercise tolerance; on the other hand, SP supplementation does not seem to enhance physical performance in power athletes.

As for the anti-inflammatory and immunomodulatory effects, the majority of the studies suggest the lack of SP benefits on muscle recovery, and despite the idea that SP supplementation may protect athletes from immune dysfunction associated with intense exercise, evidence supporting benefits on the immune system from SP supplementation is still lacking.

Therefore, evidence for promoting SP consumption in healthy subjects to improve athletic performance and accelerate recovery is still scarce; thus, at this stage, it could only be suggested in elite athletes who do not achieve the recommended antioxidant dietary intake to improve deficiencies and/or nutritional status.

Future directions

In order to better explore the impact of SP on healthy subjects involved in exercise, we recommend that research:

1. Use multiple biomarkers to assess oxidative stress or redox signaling and abandon outdated assays (i.e., TBARS and TAC).
2. Evaluate interference with training adaptation (e.g., hypertrophy, strength and mitochondrial biogenesis).
3. Assess benefit on muscle damage, and recovery in real life or similar conditions (e.g., during a competition, high-intensity training period or consecutive simulated games).
4. Evaluate impact on prevention or treatment of URS, especially in winter.
5. Contemplate the use of a human systems biology approach with multi-omics outcomes to have a system-wide overview of the adaptive response to exercise and nutritional interventions.
6. Explore the ergogenic aids in female athletes.

Data availability statement

The original contributions presented in this study are included in the article, further inquiries can be directed to the corresponding author.

Author contributions

PC, VDO, and FG: conceptualization. PC, GC, and FG: methodology. PC, MD, GC, and FL: formal analysis. PC, GC, and MD: data curation. PC and GC: writing—original draft preparation. PC, GC, MD, FL, VDO, FG, and GL: writing—review and editing. VDO, FG, and GL: supervision. GL: funding acquisition. All authors read and agreed to the published version of the manuscript.

Funding

This work was supported by the Italian Ministry of University and Research – Operative Research Programme "Research and Innovation" 2014–2020 under grant ARS01_01166, project "Development of Nutraceutical Products from Natural Sources – BIONUTRA".

Acknowledgments

We thank Emily Riley for the English revision of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Article *in* Medicine and Science in Sports and Exercise · January 2010

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Ergogenic and Antioxidant Effects of Spirulina Supplementation in Humans

MARIA KALAFATI[1,2], ATHANASIOS Z. JAMURTAS[1,2], MICHALIS G. NIKOLAIDIS[1,2], VASSILIS PASCHALIS[1,2], ANASTASIOS A. THEODOROU[1,2], GIORGOS K. SAKELLARIOU[1,2], YIANNIS KOUTEDAKIS[1,2,3], and DIMITRIS KOURETAS[4]

[1]Institute of Human Performance and Rehabilitation, Center for Research and Technology – Thessaly, Trikala, GREECE; [2]Department of Physical Education and Sport Science, University of Thessaly, Trikala, GREECE; [3]School of Sport, Performing Arts and Leisure, Wolverhampton University, Walshall, UNITED KINGDOM; and [4]Department of Biochemistry & Biotechnology, University of Thessaly, Larissa, GREECE

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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Submitted for publication November 2008.
Accepted for publication April 2009.

0195-9131/10/4201-0142/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/MSS.0b013e3181ac7a45

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

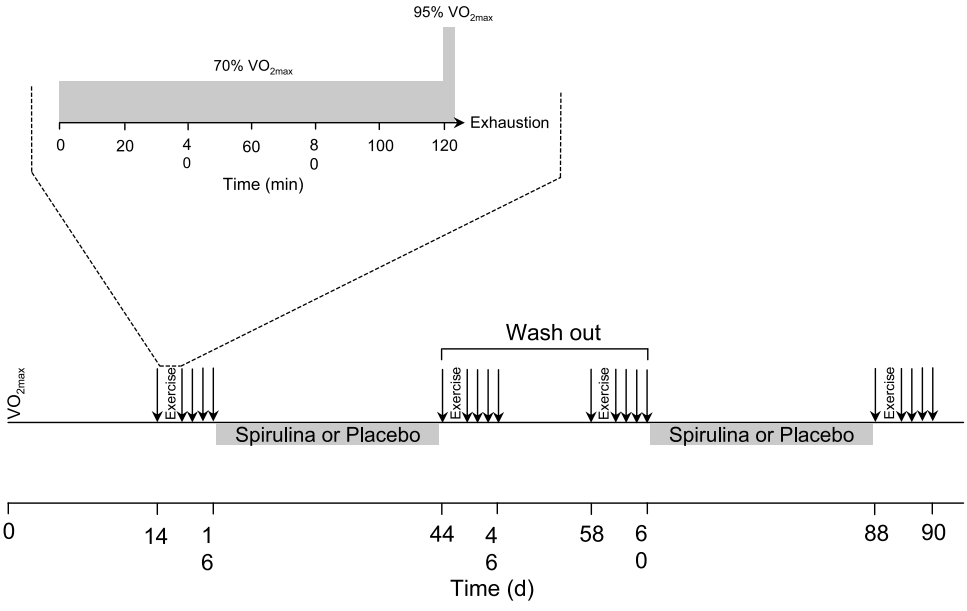

FIGURE 1—Study design. ***Arrows*** **indicate blood sampling.**

$\dot{V}O_{2max}$ 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 $\times$ 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 $\times$ 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.

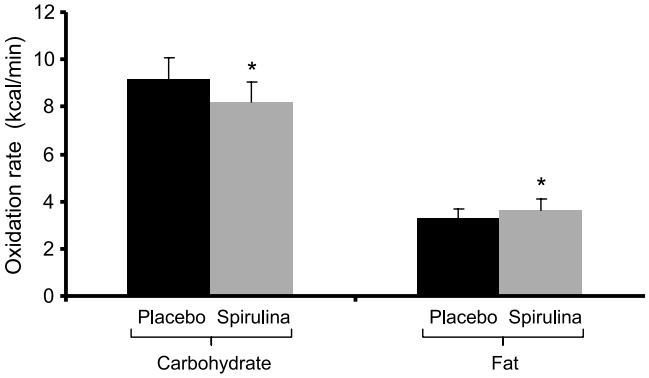

FIGURE 3—Oxidation rate in the placebo and spirulina trial during the 2-h run (mean ± SEM). *The carbohydrate and fat rates were significantly different between the placebo and the spirulina trials ($P < 0.05$).

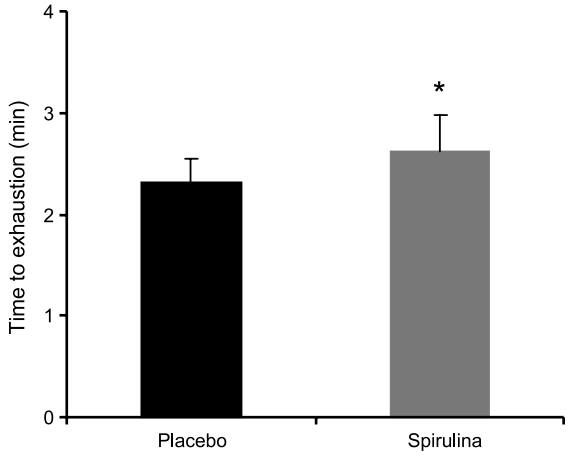

FIGURE 2—Exercise performance at 95% $\dot{V}O_{2max}$ in the placebo and spirulina trial (mean ± SEM). *Significantly different from the placebo trial ($P < 0.05$).

DISCUSSION

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

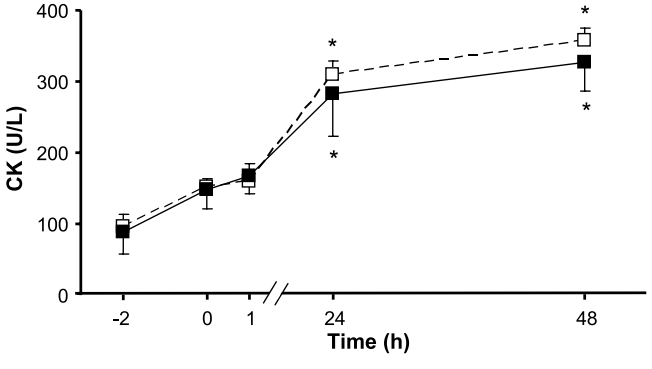

FIGURE 4—Creatine kinase (CK) activity in the placebo (*open rectangles*) and spirulina exercise trials (*filled rectangles*; mean ± SEM). *Significantly different from the resting value in the same trial ($P < 0.05$).

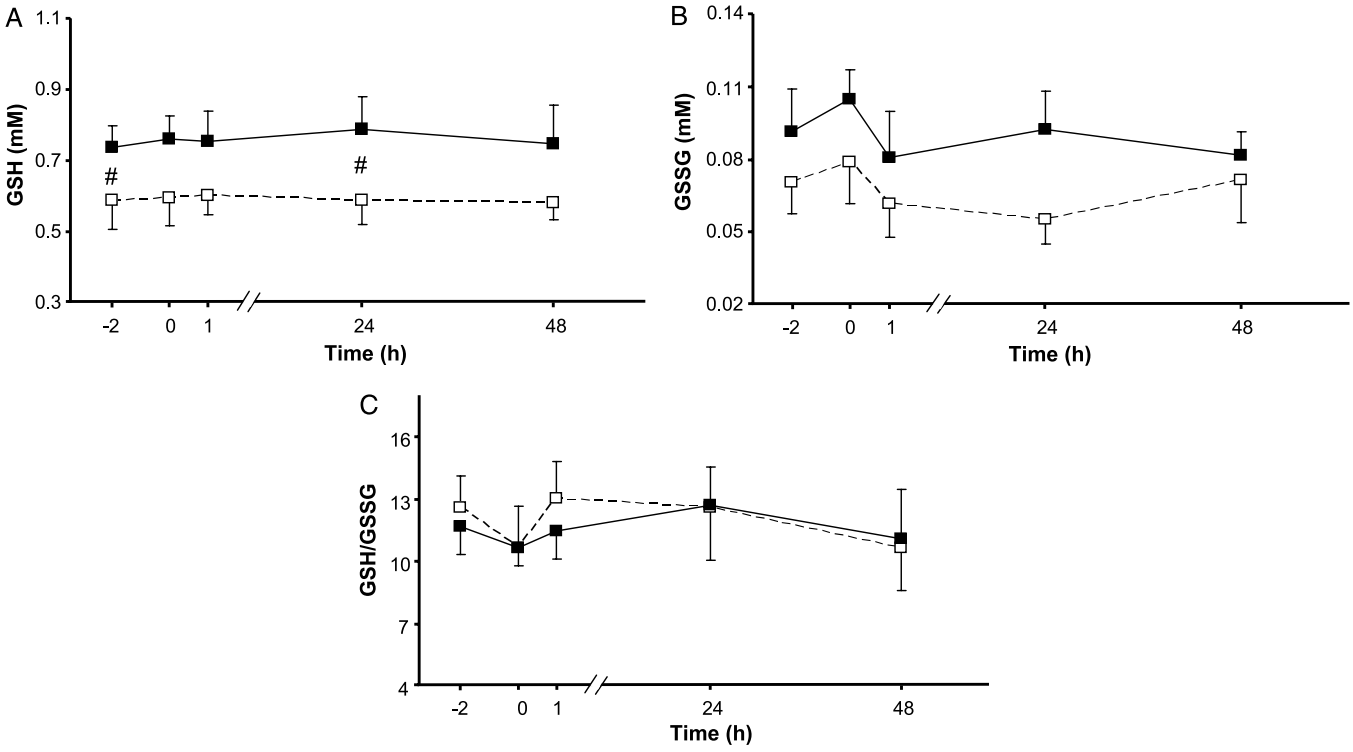

FIGURE 5—GSH (*A*) and GSSG concentrations (*B*) as well as GSH/GSSG (*C*) ratio in the placebo (*open rectangles*) and spirulina exercise trials (*filled rectangles*; mean ± SEM). #Significantly different between placebo and spirulina trial at the same time point ($P < 0.05$).

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).

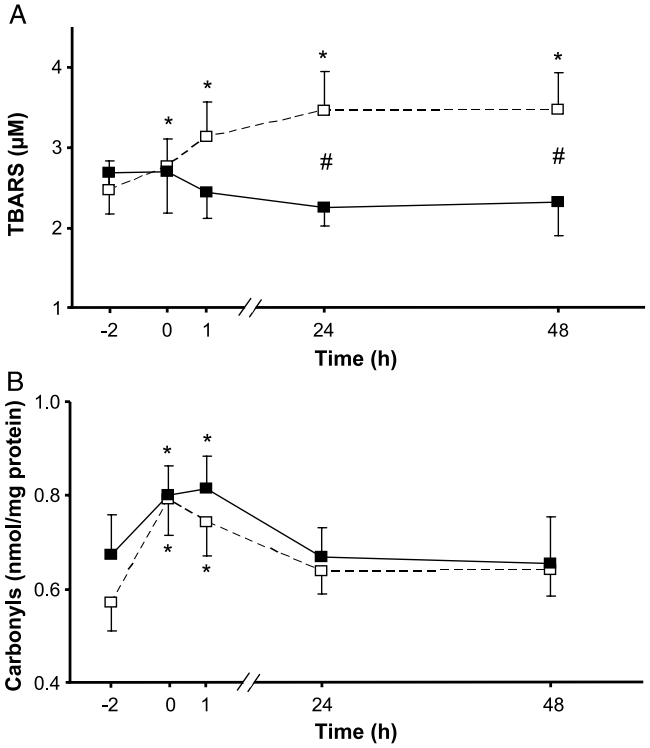

FIGURE 6—TBARS (*A*) and protein carbonyl (*B*) concentrations in the placebo (*open rectangles*) and spirulina exercise trials (*filled rectangles*; mean ± SEM). *Significantly different from the resting value in the same trial ($P < 0.05$). #Significantly different between placebo and spirulina trial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

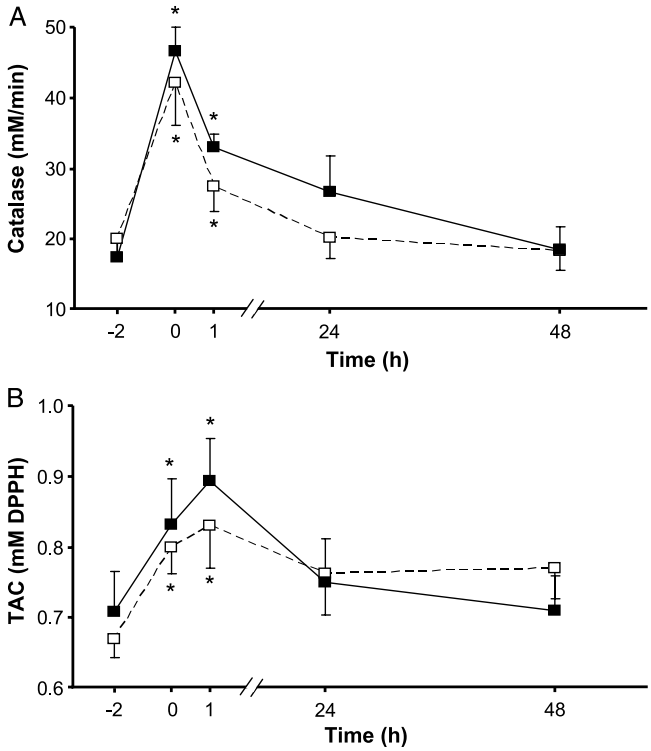

FIGURE 7—Catalase activity (*A*) and total antioxidant capacity (*B*) in the placebo (*open rectangles*) and spirulina exercise trials (*filled rectangles*; mean ± SEM). *Significantly different from the resting value in the same trial ($P < 0.05$).

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.

This study was supported by funds from the Center of Research and Technology – Thessaly.

The results of the present study do not constitute endorsement by American College of Sports Medicine.

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