# SPIRULINA DOES NOT DECREASE MUSCLE DAMAGE NOR OXDIDATIVE STRESS IN CYCLING ATHLETES WITH ADEQUATE NUTRITIONAL STATUS

Accepted for publication 02.07.2010

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**ABSTRACT:** The objective of this study was to assess the effect of Spirulina maxima on oxidative stress and muscle damage in cycling athletes subjected to high volume and intensity of training. Eighteen male athletes were randomly divided into an experimental group (n=11) with age 27.8±3.5 and placebo (n=7) with age 34.3±2.3 in a double-blind fashion. They carried out a protocol of Spirulina dietary supplementation (7.5 g/day) of placebo for four weeks and maintained their trainings during this period. A nutritional anamnesis was performed and blood tests were done to determine pre and post levels of creatine kinase (CK), lactic dehydrogenase (LHD), superoxide dismutase (SOD) and malondialdehyde (MDA). The supplemented and placebo groups performed the same volume training, has adequate macronutrients and antioxidant vitamins ingestion before study, as well as initial CK, LDH, SOD and MDA levels. Supplementation did not promote a significant alteration in CK levels on supplemented group (158.4±16.3 for 140.0±16.6 U/l, p>0.05), LDH (420±13.2 for 394.9±27.9 UI/l, p>0.05), MDA (2.8±0.2 for 2.9±0.4 nmol/ml, p>0.05), nor an increase in the SOD (7.3±0.6 for 7.0±0.6 U/mg Hb, p>0.05). We conclude that administration of Spirulina does not interfere in the magnitude of muscle damage nor in antioxidant status of cycling athletes that practice intense training.

**KEY WORDS:** exercise, spirulina, oxidative stress, muscle damage, athletes, dietary supplementation

## INTRODUCTION

Spirulina is a blue-green algae with a high protein content (65 to 70% of its dry weight), all the essential amino acids, rich in vitamins, minerals and all the essential fatty acids. It is 15% complex carbohydrate and has a great number of antioxidant substances, of which beta-carotene, vitamin E, oligoelements and an uknown number of bioactive substances [8, 12, 26].

Many animal and human studies have provided possible beneficial effects of the Spirulina under several diseases such as arterial hypertension and dyslipidemias [10], cancer [7], leukemia and anemia caused by cadmium intoxication [22], and kidney damage [1]. Many of these benefits are due to its antioxidant activity, increase in the synthesis of endothelial nitric oxide and immunosuppressive potential of the Spirulina [4, 8, 18, 20].

Due its nutritional, immunosuppressive and antioxidant properties, Spirulina can provide protection also in athletes. High volume and intensity of physical training imposes a challenge for athletes to modulate their immune system, as well promotes increase the of reactive oxygen species (EROs) production in animals and human [5, 11, 16, 19, 23]. Intense and voluminous training also promotes important alterations in protein metabolism, which can result in catabolic reactions, especially in the presence of an insufficient and inadequate diet of proteins, carbohydrates and calories [23, 24, 25].

Spirulina is already included in the daily diet of some athletes [9]. However, few studies were conducted to confirm the ergogenic effect of these algae on sport context. Then, the relevance of this investigation is to evaluate the possible ergogenic effect of the Spirulina for athletes. Indeed, other immunosuppressive and antioxidant supplementation is already used to attenuate oxidative stress and muscle damage caused by the high loads of training to which the athletes are constantly subjected [15].

In view of this, this study was to test the hypothesis that Spirulina maxima supplementation can reduce oxidative stress and muscle damage in cycling athletes subjected to high volume and intensity training regimes.

## MATERIALS AND METHODS

*Study subjects and ethical questions*: the study was carried out with 18 cyclist athletes of regional level, week frequency of training of six sessions /week and estimate duration per session of two to six hours. They were divided into two groups: experimental group (n=11), with an mean age of 27.8±3.5 and a placebo group (n=7) with an mean age of 34.3±2.3. The subjects were randomly allocated and Spirulina and placebo groups. It was a double-blind study. The Research Project was approved by the Research Ethics Committee, of the Health Science Center in the Federal University of Paraíba, protocol number 00273. All the participants were previously informed of the objectives of the Research and signed a free and informed consent.

*Design of the study*: initially, a nutritional anamnesis, body composition assessment, functional evaluation (ergoespirometric test) and blood test to determine the serum levels of creatine kinase (CK), lactate dehydrogenase (LHD), superoxide dismutase (SOD) and malondialdehyde (MDA) were performed. Then, the subjects started a period of supplementation with Spirulina or placebo for 4 weeks, administered following a double blind model. 24 hours after this period, a new blood test was performed to determine the same variables. The participants continued their training routines during the study period, but all sessions were registered. Their eating habits were monitored and restricted the use of all nutritional supplements, except supplementation of carbohydrates during training.

*Functional and nutritional evaluations:* Ergoespirometric test was performed to evaluate peak VO2 and anaerobic threshold. It was performed according Balke protocol. Three 24 hour food recordatory was used weekly. Analysis of the recordatory was performed with the NutWin software (version 1.5 – 2002). To characterize habitual diet and consumption of antioxidant substances, an mean value was obtained from the three recordatory performed.

*Subject preparation:* One week before the study, all athletes performed a supplements washout and maintained this conduct until the end of the study. However, following the athletes requests, they were allowed to continue carbohydrate supplementation, but only during training sessions and limited to an amount of 0.3 to 0.5 g/Kg of body mass /hour. Three days before two blood collection, the athletes progressively reduced their training load, in such a manner that no training was performed 24 hours before this procedures. Likewise, they abstained from training one day before of the ergoespirometric test.

*Spirulina and placebo preparation and supplementation*: Spirulina and placebo were prepared in a manipulation laboratory (Dilecta, João Pessoa-PB, Brazil). Spirulina was prepared from the extract of native algae, while the placebo was made from corn starch. The subjects consumed 7.5g of the substances per day (29.6 Kcal of Spirulina and 26.25 Kcal of placebo). It was divided in 4g in the morning and 3.5g in the afternoon, for a period of four weeks. The safety dosage recommended for consumption of Spirulina is up to 10g daily [6]. Capsules with Spirulina or placebo were delivered daily at the home or work address of the athletes. The quantity destined to be consumed in the morning were done in the presence of the person responsible for distribution, while the subjects were trusted to consume the other capsules in the afternoon.

**TABLE I.** PHYSICAL CHARACTERISTICS OF THE ATHLETES' TRAINING AND CONSUMPTION OF CALORIES AND MACRONUTRIENTS.

| Characteristics | Experimental (n=11) | Placebo (n=7) |
|---|---|---|
| Age (years) | 27.8 ±4 | 34.3±3 |
| Body mass (kg) | 69.2±3 | 76.3±3 |
| Height (cm) | 1.73±0.02 | 1.75±0.02 |
| Body Mass Index (kg/m²) | 23.1±1 | 24.9±1 |
| Percentage of body fat (%) | 8.6±1 | 12.1±2 |
| Peak $\dot{V}O_2$. (ml/kg.min.) | 57.7±2 | 55.2±1 |
| Anaerobic limit (% of the peak $\dot{V}O_2$) | 73.2±5 | 69.5±3 |
| Mean time of training in life (years) | 6.7±1 | 5.6±0.7 |
| Avereage time of training in the season (years) | 11.2±0.3 | 11.6±0.4 |
| Total energetic level (kJ) | 12272±1251 | 12083±879 |
| Carbohydrates (g/kg/d)* | 6.3±0.5 | 5.5±0.4 |
| Proteins (g/kg/d) | 2.2±0.2 | 1.9±0.1 |
| Lipids (g/kg/d) | 1.0±0.1 | 0.9±0.2 |

Note: The data represent the mean and the mean standard error.
* Food consumption considering the consumption of supplements.
No statistical differences were found among the groups.

**TABLE 2.** CONSUMPTION OF ANTIOXIDANTE SUBSTANCES BY THE ATHLETES.

| Antioxidants | Experimental (n=11) | Placebo (n=7) |
|---|---|---|
| Vitamin A (UI) | 2850±731 | 3288±816 |
| Vitamin C (mg) | 222±48* | 216±56* |
| Vitamin E (ATE) | 3.5±0.6* | 4.6±0.7* |

Note: Recommended daily consumption: Vitamin A: 3300 UI; Vitamin C: 75 mg; Vitamin E: 15 ATE; * Indicates the statistical difference in comparison to the alleged (RDA), $p<0.05$; The data represente the mean and the mean standard error.

On the following day, the researchers always confirmed the ingestion in the afternoon.

*Training protocol*: The subjects were at the end of the season, with 10 months of uninterrupted training. During the four weeks of the study, they did not participate in competitions and were told to maintain their trainings as usual. Training was performed six times a week, with a week volume of 490 Km in 16 hours and intensity which varied according to sessions. No alterations occurred in the volume nor intensity during those weeks. Subjects were told to keep a daily recording of training sessions (Table 3).

*Blood analyses*: The collections before and after supplementation were performed between 5 and 7 AM, and the subjects were told to fast for 8 to 12 hours. A 10ml volume of blood were collected from the antecubital vein and separated into serum tubes (measurement of CK, LDH and MDA) or heparinized tubes (to SOD measure). Twenty minutes after collection, the samples were centrifuged for 15 minutes at 3000 rpm. Analyses of CK and LDH were performed immediately after centrifugation. The blood samples for analysis of MDA and SOD were immediately frozen in liquid nitrogen and maintained at -80º C until analysis, which was performed 5 days after collection of the blood.

**TABLE 3.** VOLUME AND INTENSITY OF THE ATHLETES' TRAINING.

| | Distance traveled (km) | | Mean speed (km/h) | | Duration (h) | |
|---|---|---|---|---|---|---|
| | Experimental | Placebo | Experimental | Placebo | Experimental | Placebo |
| Monday | 56±1 | 58±1 | 29±0.3 | 28±0.4 | 1.8±0.06 | 1.9±0.07 |
| Tuesday | 38±2 | 40±2 | 37±1.0 | 36±1.3 | 0.9±0.06 | 1.0±0.09 |
| Wednesday | 117±4 | 123±5 | 29±0.4 | 26±0.9 | 3.7±0.07 | 4.0±0.07 |
| Thursday | 74±2 | 76±3 | 29±0.9 | 29±0.9 | 3.0±0.04 | 2.7±0.1 |
| Friday | 41±2 | 40±3 | 27±0.9 | 26±1.0 | 1.0±0.1 | 1.0±0.1 |
| Saturday | 148±4 | 152±5 | 29±0.6 | 28±0.8 | 4.8±0.2 | 5.0±0.3 |

Note: The data represent the mean and the mean standard error. No statistical differences were found for the distance traveled, mean speed and duration of trainings.

**TABLE 4.** SERUM LEVEL VALUES OF CREATINE KINASE (CK), LACTIC DEHYDROGENASE (LHD), MALONDIALDEHYDE (MDA) AND SUPEROXIDE DISMUTASE (SOD) IN THE EXPERIMENTAL GROUP (SPIRULINA) AND PLACEBO, BEFORE AND AFTER 4 WEEKS OF INTERVENTION.

| Variable | Before | After |
|---|---|---|
| CK (U/L) | | |
| Spirulina (n=10) | 158±16 | 140±17 |
| Placebo (n=7) | 141±11 | 145±13 |
| LDH (U.I./L) | | |
| Spirulina (n=11) | 420±13 | 450±30 |
| Placebo (n=7) | 395±28 | 386±16 |
| SOD (U/mg Hb) | | |
| Spirulina (n=11) | 7.3±0.6 | 7.3±0.5 |
| Placebo (n=7) | 7.0±0.6 | 6.9±0.5 |
| MDA (NMOL/ML) | | |
| Spirulina (n=11) | 2.8±0.3 | 2.4±0.2 |
| Placebo (n=7) | 2.9±0.4 | 2.3±0.2 |

Note: The data represent the mean and the mean standard error. No statistical differences were found for the distance traveled, mean speed and duration of trainings.

*Analysis of CK and LDH*: Analysis was made by the UV kinetic method, with Biosystems commercial kits (Biosystems SA, Espanha). The reading was performed in Beckman Synchron CX5 automated espectrophotometer (Beckman Colter, Inc., Fullerton, CA, USA), at wave lenghts of 340 nm and 500 nm, for CK and LDH, respectively.

*Analysis of SOD and MDA*: The Ransod commercial kit (Crumlin, CO. Antrim, United Kingdom) was used to analyze SOD activity. A volume of 0.5 ml of blood was centrifuged at 3000 rpm for 10 min and then the plasma was aspirated. Next, the erythrocytes were washed four times with NaCl at 0.9%, and centrifuged for 10 minutes at 3000 rpm after each wash. Subsequently, 2 ml of cold deionized water was added and maintained at 4º C for 15 minutes. Finally, the lysate was diluted 25 times with 0.01 mol/l of phosphate buffer pH 7.0. The preparation and addition of reagents were done according to the manufacturer's instructions. The reading was performed in a Beckman DU 640 espectrophotometer (Beckman Colter, Inc., Fullerton, CA, USA), under a wave length of 505 nm and at a temperature of 37° C. The SOD activity was expressed as units per miligrams of hemoglobin (U/mg Hb)

Determination of MDA was done through the colorimetric method. The color reagents, Sulphate Sodium 1.5M, H2SO4 3M, 100% Thrichloroacetic Acid (TCA), standard MDA and serum pool control samples were prepared in the laboratory. N. Butylic alcohol and Saline solution as well as the precipitant for LDL was obtained from Labtest (Lagoa Santa, Minas Gerais, Brazil). The reading was performed with the supernatant in the espectrophotometer under wave length of 530 mm. The results were expressed in nmol/ml.

*Data analyses:* Data are presented as mean and standard error of mean. The data were previously tested for normality, which indicated the possibility of using parametric tests in the analyses. The unpaired T-test was performed for comparison of the physical, functional and nutritional characteristics between groups. Comparisons of pre and post supplementation values were made through ANOVA test. A margin of error of 5% was adopted for all tests. The Instat software, version 3.03, was used (GraphPad, San Diego, CA, USA).

## RESULTS

The physical and functional characteristics were similar among experimental and placebo groups at begin of study. For nutritional aspects, all the subjects presented a normoglycemic (considering the consumption of carbohydrate supplements), normolipidemic and hyperproteinemic diet according to Brazilian Association of Exercise and Sports Medicine [3] (Table 1). Both groups were statistically similar in all the nutritional variables. Consumption of antioxidant vitamins is found in table 2. The athletes presented a high mean of food consumption of vitamin C and normal consumption of vitamin A compared to the RDAs. Consumption of vitamin E was deficient in all of the study's subjects, regardless of the group to which they pertained.

During the study, the athletes performed four microcycles (weeks of training) with an mean volume of 474 km per week for the experimental group and 488 km for the placebo group. Table 3 presents relevant data regarding the volume and intensity of the exercises performed. During the four weeks, the athletes repeated the same training.

Before starting supplementation, the subjects in both groups had similar values of muscle enzymes CK and LDH, and both were within the normal range for athletes. The same occurred with regards to antioxidant status, for similar pre-supplementation values of SOD and MDA were observed in the groups, a day before the start of the experimental protocol and within normal range. The supplementation protocol with Spirulina was not able to decrease serum levels of CK or LDH. Likewise, the antioxidant status was not altered by consumption of Spirulina or placebo (Table 4).

Before the start of the study, four of the 11 subjects in the experimental group presented levels of CK above the normal range. (213.7±13.2 U/L). They presented a reduction of 23%, while only a reduction of 9.5% was observed in the others. However, even this greater reduction was not statistically significant.

## DISCUSSION

Spirulina properties have already been studied in the context of prevention and treatment of various diseases. These studies demonstrated that some of the algae's nutrients have antioxidant properties [21, 26]. Lu et al.[14], demonstrated positive changes in the plasma concentrations of MDA, SOD, LDH, glutathione peroxidase and lactate, besides the preventive effect on skeletal muscle damage, after supplementation of Spirulina for three weeks in humans. However, this estudy involved non trained subjects and only one session of exercise. Kalafati et al [13], demonstrated increase in time to fatigue and a bets antioxidant status in moderately trained males. However, only one bout of exercise composed the protocol of this study. Indeed, the protective effect of Spirulina to long term training induced overtraining or overreaching is still unclear, although Milasius et al. [17] have found quantitative improvement in immunological parameters after 14 days of supplementation with spirulina, which persisted even after cessation of supplementation. Even though these data are still incipient, Spirulina is already being commercialized as a nutritional supplement, with promises of ergogenic activity for practitioners of physical exercise. However, as far as we know, this is the first study where the ergogenic effects of Spirulina were assessed in athletes that training at high volume and intensity loads.

Despite these considerations, the study's data clearly demonstrated that Spirulina supplementation did not have any ergogenic effect on the assessed athletes, at least from the perspective e of protecting against proteolysis and oxidative stress. A possible explanation for the results was that athletes had an adequate nutritional status regarding to macronutrients. From the viewpoint of antioxidant vitamins, the subjects presented deficient values only of vitamin E. Moreover, we must consider that, even though all the athletes were in the final phase of an 10 month season under high volumes and intensity of training, the levels of enzymes marking muscle proteolysis (CK and LDH), as well as antioxidant status' markers (SOD and MDA) were normal.

The period of administration of Spirulina in this study was longer than in the study conducted by Lu et al. [14], in which authors observed a protective effect on muscle damage and oxidative stress resulting from one session of progressive exercise in untrained subjects. Daily consumption of Spirulina in other studies varied from 3 to 7.5g [2, 13, 14]. The present study made use of the highest amount ever used, 7.5g, and the maximum safety dosage, 10g [6]. Therefore, this procedures eliminates the argument that the absence of effects could be due to low amounts and short period of administration of Spirulina.

Although athletes at a high competitive level, training six times a week, with an mean training volume of 474 km (experimental group) were chosen, they entered the study with mean values of CK, LDH, SOD and MDA within normal range. This may be one of the reasons for the lack of ergogenic effects of the supplement under study. To test this possibility, five athletes with more elevated CK values were compared to others. They presented reduction in CK when supplemented with Spirulina, but this reduction was not statistically significant (data now shown). The same occurred with athletes who had a SOD mean close to the inferior limit. Even then, supplementation did not improve the antioxidant status of these athletes.

Thus, it can be concluded that in cycling athletes without previous oxidative stress caused by training and with food habits deficient in vitamin E but adequate in other nutrients, supplementation with Spirulina does not have any ergogenic effects with regards to protection against chronic muscle proteolysis and oxidative stress induced by high volume and intensity of training.

Considering the previously reported benefits of Spirulina in athletes with unbalanced nutritional or training conditions, the ergogenic effects may become visible. However, to avoid commercial speculations of this algae as a nutritional supplement, it is preferable to await further studies before recommending supplementation of Spirulina in sports.

## CONCLUSIONS

Despite high concentration of proteins and antioxidants in Spirulina, supplementation was not able to alter the magnitude of muscle damage or able to reduce oxidative stress in cycling athletes with adequate nutritional status, who perform a high volume of training.

**Acknowledgements**

This study was supported by CAPES- Education ministry of Brazil.

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