Effects of Spirulina on Inflammation and Fatigue

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

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The Ohio State University

2014

Master's Examination Committee:

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Abstract

Background:
Spirulina is filamentous blue green microalgae with a vast array of beneficial ingredients (1). Spirulina could lead to a decrease in inflammation as well as physical and mental fatigue. However, the amount of information in the literature is still limited. This randomized double blinded clinical trial addressed inflammation and fatigue in 37 participants.

Methods:
This IRB approved study enrolled 37 participants who were randomly assigned to either the placebo or the treatment arms of the study. There were 17 males and 20 females between 20 and 44 years of age. Twenty participants were randomized to the placebo group while 17 were randomized to the treatment group administered 3 grams of Spirulina per day. To understand the effects of Spirulina on mental fatigue, an Uchida-Kraepelin test (UKT) as well as a daily life mental fatigue test were administered and analyzed. To understand the effects of Spirulina on physical fatigue, distance and calories burned in 30 minutes were
recorded and analyzed. To address the effects Spirulina has on inflammation, blood samples were taken at baseline and at the end of supplementation. C reactive protein was run on these samples as a marker for inflammation.

Results:
The physical fatigue data was numerically described using kilocalories and miles over a 30 minute period on a cross training elliptical machine. There was no significant change seen over time in kcals/mile in the placebo or the supplement group. However, there was a significant difference between the treatment group and the placebo group using an unpaired t test analysis in miles, kilocalories, and kilocalories/mile. The Mental fatigue test (UKT) test showed a significant change over time between the final day compared to the baseline in the treatment group but not in the placebo group. However, the unpaired t test showed no significant difference between the treatment group and the placebo group. The multi dimensional fatigue questionnaire filled out by participants at baseline, at the start of supplementation, and after 4-5 weeks of taking the supplementation showed a significant reduction in fatigue in the treatment group over the course of the study among men only. However, there was no significance seen among just women or in the entire group. The CRP inflammatory blood markers did not show a significant difference between the Spirulina and the placebo group. In
addition, there was no significant change seen in the CRP levels over time in the treatment group.

**Conclusions:**

In a small randomized clinical trial of healthy individuals, Spirulina supplementation at a dosage of 3 grams per day improved measures of mental and physical activity compared to placebo. However, there were no significant changes seen in the C Reactive Protein (CRP) inflammatory blood markers.
Acknowledgments

First and Foremost, I would like to give all thanks and glory to The Almighty God.

I would like to express my sincere gratitude to my Mom who has always been and continues to be such an inspiration and support to me. Thank you for everything.

I would also like to thank Lauren Hassinger and Joshua I. Davis for their assistance throughout this research project and Causenge Cangin for her assistance with the statistical analysis. You have contributed so much to this study and your time and effort is valued and greatly appreciated.

Finally, I would like to thank the members of my committee: The chair of my committee, Dr. Disilvestro and committee members Dr. Harris and Dr. Bomser. You have provided a wide array of support and guidance through this process. I would like to thank you for this opportunity and for your time and dedication.
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Publications


**Fields of Study:**

Major Fields: Human Nutrition
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Chapter 1: Introduction

Spirulina is a filamentous blue green microalgae (cyanobacterium) and consists of spiral filaments (1,2). The growth of Spirulina occurs in alkaline water environments in America, Mexico, Asia and Central Africa (1). Spirulina has a vast array of nutritional benefits and is consumed in a variety of cultures worldwide (1).

Spirulina has a complex chemical makeup that contains a variety of vital components such as proteins, amino acids, vitamins, minerals, fatty acids and polysaccharides (2,3). Newer understanding of Spirulina has changed its classification from the plant kingdom to part of the bacteria kingdom (1). There are three species of Spirulina that are edible for consumption; Spirulina platensis, Spirulina maxima and Spirulina fusiformis (1). These three species are the most common types of Spirulina investigated in the scientific literature (1). Spirulina is considered to be safe to ingest as long as the purity of the supplement is maintained (1). However, there have been reported cases of adverse side effects due to contamination of supplements (1).
It is important to understand the potential effects that Spirulina could have on a variety of different disease states. There have been numerous preclinical studies both in vivo and in vitro looking at the effects of Spirulina. In addition, there have been a small number of clinical human based studies. The amount of clinical information is still limited and more data is needed to understand the clinical relevance of Spirulina.

Spirulina has potential anti inflammatory, anti oxidant and chemo protective effects (1). It has also been studied and has potential effects in a wide variety of disease states including but not limited to cardiovascular disease, cancer, infection, chronic fatigue syndrome, allergic rhinitis, and diabetes. In addition, Spirulina can help to inhibit exercise stimulated inflammation and oxidative stress, which may help reduce fatigue during exercise (4, 5).

**Hypothesis:** Spirulina contains a vast array of beneficial ingredients which could lead to a decrease in inflammation as well as physical and mental fatigue.

Specific Aim 1: To understand the effects of Spirulina on physical fatigue, distance and calories burned in 30 minutes while exercising on a cross training machine was recorded and analyzed.
Specific Aim 2: To understand the effects of Spirulina on mental fatigue, an Uchida-Kraepelin test (UKT) as well as a daily life mental fatigue test was administered and analyzed.

Specific Aim 3: The anti-inflammatory effects of Spirulina was examined by blood work pre and post intervention.

The blood test chosen for this study will address the anti-inflammatory effects of Spirulina. C-reactive protein (CRP) is a protein produced by the liver (6). It is a broad measure of inflammation in the body but will not determine the specific site of inflammation (6). CRP can also be used to estimate risk of cardiovascular disease and in some studies have been found to be more effective than cholesterol as a predictor of heart disease (6). In addition, CRP can be a marker of disease risk for a variety of different chronic disease states (6). These include but are not limited to cancer, rheumatoid arthritis, and inflammatory bowel disease (6). This blood marker will help determine the anti-inflammatory effect of Spirulina.
Chapter 2: Methods

The protocol for the study was approved by the Biomedical Sciences Institutional Review Board (IRB) for research on human subjects of The Ohio State University. Forty healthy males and females were recruited for participation. Potential participants were given a questionnaire to screen for eligibility criteria. The exclusion criteria included: Tobacco use, pregnancy, current use of Spirulina, known chronic disease, and/or current training for a marathon or other strenuous exercise regimen. The inclusion criteria included: Ability to maintain exercise for 30 minutes and willingness to take the supplementation and comply with the aspects of the trial. Thirty eight participants between 20 and 44 years of age were selected and consented to enroll. One participant discontinued with the study leaving 37 participants. There were a total of 17 males and 20 females. The participants were randomly selected for placebo or supplementation (group A and group B). Group A contained 8 males and 12 females for a total of 20 participants while group B contained 9 males and 8 females for a total of 17 participants.
The treatment group was administered 3 grams of Spirulina per day which was administered at 6 x 500 mg tablets per day. The participants in the placebo group were given a gelatin capsule. All subjects were asked to take the tablets each day with a meal containing fat to help with the absorption of some of the components of Spirulina.

The timeline of the study included an initial day/baseline where participants signed a consent form, were given the Piper fatigue scale test (evaluation of general sense of fatigue), the mental fatigue (UKT test) and a cross training machine assessment for baseline physical fatigue. On the first day of supplementation, the participants took the first supplement or placebo with a meal about 4 hours before coming to the research site. Mental fatigue test and cross training machine assessment were conducted and data was collected. An intermediate assessment of physical fatigue was conducted approximately one week after starting supplementation. The participants took the supplement or placebo from approximately day 10-64. On the final day of supplementation, the participants took the last supplement or placebo before coming to the facility. The final mental and physical fatigue tests as well as blood work was conducted.
Testing Utilized:

Piper fatigue scale test: Assessment of a subject’s self perception of general fatigue. This study used a modification known as the Multidimensional Assessment of Fatigue (MAF). The scale contains 16 items and measures four dimensions of fatigue: severity, distress, degree of interference in activities of daily living, and timing. The evaluations use numerical rating scales and two have multiple choice questions. The score is the sum of all the numbers circled.

Mental fatigue test: the Uchida-Kraepelin test (UKT). This is a computer test and involves a series of calculations of random numbers for 15 minutes time. The number of correct answers for each minute is used for data reporting.

Blood Tests performed:

C reactive protein (CRP): measured using an ELISA kit from Invitrogen
Biostatistical Methods of Data Analysis:

Statistical Analysis Software (SAS) was utilized for T test analysis and Generalized Estimating Equation (GEE). Paired T tests were conducted to compare the baseline measurements to measurements over the course of the study. This was run for both the treatment and the placebo arms of the study and for each outcome measure including kcal/mile, mental fatigue score and multidimensional fatigue score. Unpaired T tests were performed to analyze the significance differences in the placebo vs. the treatment group. Time series data were plotted and tested for differences over time in the treatment vs. the placebo group using the Generalized Estimating Equation (GEE).
Spirulina and fatigue:

This double blind randomized clinical trial addresses the effects of Spirulina on everyday fatigue as well as fatigue induced by mental and physical exertion. It is important to understand the literature in this arena so that comparisons can be assessed.

In an N-of-1 randomized double blinded study addressing Spirulina and chronic fatigue, four patients were given four weeks of placebo and four weeks of Spirulina (7). Fatigue was measured on a 10 point fatigue scale to determine the difference between the supplementation and the placebo (7). There were no significant findings suggesting that Spirulina had any more effects on reducing fatigue than the placebo supplementation (7).

Another study looking at the effects of Spirulina on fatigue was conducted using a 2 hour exercise study in 9 males (5). This study showed a decrease in fatigue using Spirulina supplementation as the time to fatigue during the 2
hour run was longer in the Spirulina group than in the control (5). There was also an increase in GSH, fat oxidation and lipid peroxidation in the Spirulina group (5). The decrease in GSH also shows the potential antioxidant effects of Spirulina.

Results from similar studies show that Spirulina decreases inflammation and oxidant stress following exercise (5). In one study, 16 students took Spirulina for 3 weeks (8). The participants taking Spirulina had significantly higher time to exhaustion on a treadmill than those not taking Spirulina (8). In addition, there were differences seen in anti-inflammatory and anti-oxidant blood markers in the Spirulina arm of the study (8). These effects could help explain the delay in exercise induced fatigue in participants taking Spirulina supplementation (4, 8).

**Cardiovascular Disease**

The effects of Spirulina on cardiovascular disease seem promising from recent studies. LDL and VLDL cholesterol are atherogenic and HDL cholesterol is known to be protective against development of atherosclerotic plaques (1). Individuals with higher levels of LDL and VLDL cholesterol are at a higher risk of cardiovascular events and elevated triglyceride levels (1). Spirulina has been studied in animal pre-clinical studies with a variety of animal prototypes. Hypolipidemic effects of Spirulina have been observed in
mice, rats, hamsters and rabbits (1). Notably, a study in hamsters fed an atherogenic diet showed lower total cholesterol, LDL, VLDL, fatty streak area and unchanged HDL when Spirulina was added to the diet (19). In the rabbit study, there was a decrease in serum total cholesterol and an increase in HDL cholesterol while no changes noted in serum triacylglycerol (20). Overall, animal studies show a hypolipidemic effect by lowering serum total cholesterol, LDL and VLDL levels (1).

Clinical studies have also been performed which demonstrate the possible effects of Spirulina on cardiovascular disease. There are two notable studies with healthy individuals which demonstrated a decrease in total cholesterol and LDL, a slight decrease in triglyceride levels and no change in HDL cholesterol (1).

There are at least two studies in healthy individuals addressing the effects of Spirulina on lipid levels. (1). The first study is in healthy elderly volunteers and showed a significant decrease in total plasma cholesterol and LDL fraction in female subjects and a decrease in total cholesterol by repeated test for treatment in male subjects without an effect in LDL fraction (9). HDL was unaffected in both genders (9). The other study recruited 16 men and 20 women (18-65) who took 4.5 grams of Spirulina per day for 6 weeks (17).
There was a hypolipidemic effect seen on TAG and LDL cholesterol as well as a decrease in blood pressure levels (17).

At least four studies have been conducted looking at lipid levels in the type 2 diabetes population. These studies showed consistent decreases in LDL, VLDL, and triglyceride levels (1). Similar results were observed in specific studies looking at patients with ischemic heart disease, nephritic syndrome and hypercholesterolemia (1). The majority of data from these studies demonstrate the potential effects Spirulina could have on the prevention and management of cardiovascular disease.

**Anti-inflammatory and antioxidant effects of Spirulina:**

Inflammation and oxidative stress are thought to contribute to a variety of disease states. Oxidative stress and inflammation are considered part of the disease process of atherosclerosis, cardiac hypertrophy, heart failure and hypertension and atherosclerosis is now considered a disease of chronic inflammation(1). The effects of controlling an inflammatory environment could be invaluable in multiple pathophysiologies.

There are several animal studies in vivo and in vitro that have been conducted looking at the anti-inflammatory and anti-oxidant effects of Spirulina. The majority of these studies demonstrate that Spirulina has an anti
oxidant and anti inflammatory effect in vivo and in vitro. These pre clinical studies had a variety of inducing agents that caused oxidative stress and/or inflammation (1). A few examples of these studies will be discussed. In a study utilizing cisplatin as an inducing agent, there was a significant restoration of renal function damaged by the agent as well as a decrease in lipid peroxidation, increase in glutathione and an increase in superoxide dismutase and catalase (14). In a mouse study using cyclophosphamide and mitomycin c as the inducing agent, there was a reduction in chromosomal damage and lipid peroxidation utilizing Spirulina (18). In a study using cadmium in a rat model, there was a decrease in lipid peroxidation and an increase in antioxidant levels (15). Using Spirulina produced a nephroprotection in rats insulted by Gentamicin Sulphate by decreasing lipid peroxidation and increasing glutathione, SOD, GPX, and NO (16).

**Spirulina and cancer:**

Several pre clinical studies have addressed the possible effects of Spirulina in cancer. Most notable are two animal studies which addressed the effect of Spirulina on oral cancer and breast cancer. The study on oral cancer showed inhibitory effects on oral carcinogenesis utilizing Spirulina (10). A dose of 1 g/day of Spirulina fusiformis was given for 12 months and complete regression of oral leukoplakia lesions was seen in 20/44 subjects compared to 3/43 in the
placebo group (10). After discontinuation of the supplement for approximately one year, recurrence was seen in 9/20 responders (10).

In the study addressing Spirulina and mammary carcinogenesis, arthospirulina platensis filamentous blue green microalgae (cyanobacterium) was administered to rats (11). This study showed an effect against breast cancer utilizing Spirulina (11). Morphological and histological examination showed cleared rat mammary tumors (11). In addition, there was a reduced incidence of DMBA induced breast tumors observed at the molecular level from an occurrence rate of 87% lowered to only 13% in the treatment groups (11). Inhibited cell proliferation and increased P53 and p21 were observed within 24 hours of treatment (11). Apoptosis was induced by 48 hours after treatment (11).

**Mechanisms for anti tumor potential of Spirulina**

There are several studies looking at the possible reasoning behind the potential cancer toxic and chemopreventative effects of Spirulina. In one study, the effects of Spirulina on Cytochrome P 450 enzymes were examined (12). The rats were given Spirulina daily for 5 weeks which showed an inhibition in enzymatic activities of CYP1A2 and CYP2E1 (12). There was an increase in mRNA/protein level of CYP2B1 and CYP3A1 but no enzymatic changes (12). The effects of Spirulina on CYP enzymes may shed some light
on the chemo preventive effects of SP on mutagenesis and carcinogenesis (12).

Another important animal study using a dog and mouse model showed that Spirulina may also have a chemoprotective and radioprotective effect (13). This is important as these are two of the most common forms of cancer treatments with the possibility of significant side effects (13). Cyclophosphamide (chemotherapy) and irradiation were used to cause hemopoietic damage in mice and dogs (13). When given Spirulina, mice showed an increase in the level of white cells in the blood and nucleated cells as well as an increase in the DNA in bone marrow (13). However, there were no effects seen on red cells or hemoglobin levels (13). In dogs, there was an increase in red cells, white cells, and hemoglobin levels in the blood and in the bone marrow (13).

A recent study published in 2013 showed Spirulina maxima was found to inhibit human cancer cells dependent on concentration (22). In addition, this study looked at the difference in potency between Spirulina grown in Deep Sea Water vs. Spirulina grown in conventional sea water (22). The paper found that S. maxima suppressed Bcl3 expression in A549 cells (22). In addition, Cytokines IL-6 and TNF alpha from B cells as well as NK cells were found to be higher in the deep sea water extract (22). Analysis showed that
the DSW had higher ratios of minerals that helped in the biosynthesis of B-carotene and ascorbic acid which contributed to the higher potential of the deep sea version (22). Two more recent studies demonstrate some additional mechanisms of Spirulina. The first study showed a secondary metabolite of cyanobacteria is hierridin B which has anti tumor effects (23). Specifically, the authors demonstrate the effect against HT-29, which is a line of colon adenocarcinoma cells (23). The second study used anti tumor assay in vitro and was conducted using 24 strains of cyanobacteria against two murine cancer cell lines colon carcinoma CT-26 and lung cancer 3 LL (24). Two extracts CYP011K and CENA69 were found to be inhibitory against the cancer cell lines (24).

**Mechanism of action against inflammation:**

Understanding the reasoning behind the effect of Spirulina on inflammation is complex, yet important to understand. It is known that Spirulina contains Phycocyanin which has significant anti oxidant and anti inflammatory properties (1). Phycocyanin is a free radical scavenger and can decrease nitrite production and suppress inducible nitric oxide synthase expression and inhibits liver microsomal lipid peroxidation (1). It also helps inhibit inflammation by decreasing the expression of COX 2 and Prostaglandin E 2
Spirulina has similar effects as it can inhibit the production of nitric oxide and PGE2 similar to Phycocyanin (1). It also inhibits NF-κB and therefore can suppress iNOS, COX-2, TNF-alpha and IL-1B (1).

A rat study was conducted using non-alcoholic steatohepatitis (NASH) model rats (21). This study showed that using Spirulina may help decrease the effects of NASH (21). These rats had an increase in plasma liver enzymes, liver fibrosis, and increase in production of ROS from the liver mitochondria from the NASH (21). Spirulina helped to prevent the progression of NASH as it lessened the inflammatory response (21).
Physical fatigue:

Physical fatigue was numerically described using kilocalories and miles over a 30 minute period on an elliptical machine. This physical fatigue test was performed at baseline, first day of supplementation, approximately one week after starting supplementation, and on the final day of supplementation. The data was analyzed comparing kilocalories and miles as individual data points as well as analyzing the rate of kcals/mile.

The paired t test was used to address the change over time in both the placebo and treatment arms. There was no significant change over time seen in kcals/mile in either of the groups (Table 1). An unpaired t test analysis was also performed addressing the difference in kcal/mile, kcals and miles between the treatment and the placebo groups (Figure 1). The treatment group as a whole performed significantly better in all three measurements (2 sided t test P value of 0.05, 0.02, 0.004 respectively) (Table2). In addition,
men in the treatment group showed significantly higher kilocalories per mile, kcals and miles than men in the placebo group when using a 1 tail T test ($p$ value from 1 sided t test =0.04, 0.006, 0.05) (Table 2). However the 2 tail T test only showed significant in the kcals ($p$=0.01). Women in the treatment group did not show a significant difference in kilocalories/mile or kilocalories but showed a significant difference in distance (miles) compared to women in the placebo group (2 side t test $p$ value= 0.04 and 1 side t test $p$ value= 0.02). (Table 2).

<table>
<thead>
<tr>
<th>Rate kcal/mile</th>
<th>2nd time to 1st time</th>
<th>3rd time to 1st time</th>
<th>4th time to 1st time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change over course of the study</td>
<td>p values (2sided)</td>
<td>P values (1side)</td>
<td>p values (2sided)</td>
</tr>
<tr>
<td>Spirulina (n=17)</td>
<td>0.526</td>
<td>0.263</td>
<td>0.093</td>
</tr>
<tr>
<td>Spirulina (n=17)</td>
<td>0.47</td>
<td>0.238</td>
<td>0.53</td>
</tr>
<tr>
<td>Placebo (n=20)</td>
<td>0.6033</td>
<td>0.698</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Table 1: Changes over time for Physical Fatigue using the rate of kcal/mile.
<table>
<thead>
<tr>
<th>Kcal/mile</th>
<th>95% Confidence Interval</th>
<th>p (2sided)</th>
<th>p (1sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both genders (n=37)</td>
<td>(-59.2, -101.93)</td>
<td><strong>0.049</strong></td>
<td><strong>0.0246</strong></td>
</tr>
<tr>
<td>Men (n=17)</td>
<td>(-94.209, 4.7688)</td>
<td>0.0755</td>
<td><strong>0.0377</strong></td>
</tr>
<tr>
<td>Women (n=20)</td>
<td>(-17.662, 19.897)</td>
<td>0.9057</td>
<td>0.5471</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Kilocalories</th>
<th>95% Confidence Interval</th>
<th>p (2sided)</th>
<th>p (1sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both genders (n=37)</td>
<td>(-117.27, -26.6472)</td>
<td><strong>0.021</strong></td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Men (n=17)</td>
<td>(-145.52, -18.788)</td>
<td><strong>0.0119</strong></td>
<td><strong>0.0059</strong></td>
</tr>
<tr>
<td>Women (n=20)</td>
<td>(-81.542, 22.717)</td>
<td>0.2612</td>
<td>0.1306</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Miles</th>
<th>95% Confidence Interval</th>
<th>p (2sided)</th>
<th>p (1sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both genders (n=37)</td>
<td>(-0.4538, -0.09042)</td>
<td><strong>0.004</strong></td>
<td>0.002</td>
</tr>
<tr>
<td>Men (n=17)</td>
<td>(-0.4778, 0.45567)</td>
<td>0.1038</td>
<td><strong>0.0519</strong></td>
</tr>
<tr>
<td>Women (n=20)</td>
<td>(-0.5392, -0.1791)</td>
<td><strong>0.0367</strong></td>
<td><strong>0.0184</strong></td>
</tr>
</tbody>
</table>

Table 2: Comparison of Spirulina supplementation versus placebo: Physical fatigue (kcal/mile, kcal, and miles)
Figure 1: Comparison of the treatment and placebo group: physical fatigue.

(Rate kcal/miles

**Mental fatigue:**

Participants were administered the computerized UKT math test at baseline, first day of supplementation and final day of supplementation. A numerical result was recorded after 15 minutes of testing. Paired T test analysis showed a significant change over time between the final day compared to the baseline in the treatment group ($p$ value $0.0254$)(Table 3). The placebo group did not
show any change over time between the baseline and final day (p value = .1552). However, the unpaired t tests show no significant difference between the treatment group and the placebo group (Table 4). This was consistent in the group as a whole, in males, and in females (p values from 2 side T test=619, .512, .118) (Figure 2).

Figure 2: Comparison of the treatment and placebo group: Mental fatigue using UKT test
Table 3: Mental Fatigue test (UKT) over time.

<table>
<thead>
<tr>
<th>T test across course of the study</th>
<th>Spirulina</th>
<th>Spirulina</th>
<th>Placebo</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mental Fatigue over time</td>
<td>p (2side)</td>
<td>p (1side)</td>
<td>p(2side)</td>
<td>p (1side)</td>
</tr>
<tr>
<td>All: 2nd time to 1st time</td>
<td>0.0775</td>
<td>0.0388</td>
<td>0.2639</td>
<td>0.132</td>
</tr>
<tr>
<td>CI=(-34.107,1.989)</td>
<td></td>
<td></td>
<td>CI=(-30.012,8.712)</td>
<td></td>
</tr>
<tr>
<td>All: 4th time to 1st time</td>
<td>0.0254</td>
<td>0.0127</td>
<td>0.1552</td>
<td>0.0776</td>
</tr>
<tr>
<td>CI=(-37.426, -2.809)</td>
<td></td>
<td></td>
<td>CI=(-28.845, 4.947)</td>
<td></td>
</tr>
<tr>
<td>Mental Fatigue</td>
<td>95%Confidence Interval</td>
<td>p (2side)</td>
<td>p (1side)</td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------------</td>
<td>-----------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>Both genders (n=37)</td>
<td>(-7.968,13.369)</td>
<td>0.618</td>
<td>0.309</td>
<td></td>
</tr>
<tr>
<td>Men (n=17)</td>
<td>(-23.425,11.793)</td>
<td>0.512</td>
<td>0.256</td>
<td></td>
</tr>
<tr>
<td>Women (n=20)</td>
<td>(-2.261,23.285)</td>
<td>0.1050</td>
<td>0.0525</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Comparison of Spirulina and placebo groups: mental fatigue

**Multi dimensional fatigue test:**

This questionnaire was filled out by participants at baseline, at the start of supplementation, and after 4-5 weeks of taking the supplementation. The test asked generalized questions about every day fatigue and a total value was calculated based on the responses. A lower total value equated to the less fatigue experienced.

Paired T tests showed significant changes in both the placebo and treatment group over time. Unpaired one tail T test show that the treatment group had a significant reduction in the multi dimensional fatigue test score among men.
(p value from 1 side T test = 0.0328 and 2 tail=.0656 ). (Figure 3). However, there was no significance seen in women or in the entire group (p value from 1 side T test=.218, .193) ( p value 2 side T test=0.387,0.193) (Table 6).

Utilizing a more robust GEE model (Generalized Estimating Equation) to address the change over time there was a significant change seen in the treatment group by the final day of supplementation. There was a significant average reduction in score by -17.93 points (p value less than 0.001)

Figure 3: Comparison of the treatment group and placebo group:
Multidimensional fatigue test.
<table>
<thead>
<tr>
<th>T test across Time</th>
<th>Spirulina</th>
<th>Spirulina</th>
<th>Placebo</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multi Dimensional</td>
<td>p (2sided)</td>
<td>p (1sided)</td>
<td>p (2sided)</td>
<td>p (1sided)</td>
</tr>
<tr>
<td>Time2 to Time1</td>
<td>0.269</td>
<td>0.8652</td>
<td>0.43</td>
<td>0.7847</td>
</tr>
<tr>
<td>Time4 to Time1</td>
<td>0.0017</td>
<td>0.0009</td>
<td>0.0011</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

Table 5 Multidimensional Fatigue test over time

<table>
<thead>
<tr>
<th>Multidimensional Score</th>
<th>95%Confidence Interval</th>
<th>p (2sided)</th>
<th>p (1sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both genders (n=37)</td>
<td>(-5.298,13.508)</td>
<td>0.3885</td>
<td>0.1943</td>
</tr>
<tr>
<td>Men (n=20)</td>
<td>(.7694,23.649)</td>
<td>0.0656</td>
<td><strong>0.0328</strong></td>
</tr>
<tr>
<td>Women (n=17)</td>
<td>(-18.609,7.836)</td>
<td>0.4173</td>
<td>0.2086</td>
</tr>
</tbody>
</table>

Table 6 Comparison of Spirulina supplementation versus placebo: Multidimensional fatigue test
**C Reactive Protein:**

There was no significant difference between the CRP levels (post intervention-pre intervention) between the treatment and placebo groups. Unpaired T Test showed a p value of .675 when comparing the two groups (95% CI= (-.2720807, .4149226). When looking at the change over time using paired T test of the treatment group, there was also no significance found (P value=0.8178) (95% CI= -.2876, .2304). GEE analysis showed that the females in the placebo group had significantly higher post CRP values than males in the placebo group (p=0.046). It also showed that the females in the treatment group had a non significant reduction in CRP levels by 0.4 (p value=0.122) (95 % CI (-.90843, .10689.)

<table>
<thead>
<tr>
<th>Gender</th>
<th>95% Confidence Interval</th>
<th>p (2side)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both genders (n=36)</td>
<td>(-.27208, .41492)</td>
<td>0.675</td>
</tr>
<tr>
<td>Men (n=16)</td>
<td>(-.7313 , .4722)</td>
<td>0.646</td>
</tr>
<tr>
<td>Women (n=20)</td>
<td>(-.33753, .6908)</td>
<td>0.469</td>
</tr>
</tbody>
</table>

Table 7 T test C Reactive Protein: Comparison of change in CRP in treatment vs. placebo
Physical fatigue:

The small amount of information available in the literature regarding the effect of Spirulina on physical fatigue lends to difficulty determining its possible effect. This trial was able to contribute some additional insight on the possible effect of Spirulina on physical fatigue.

The significant difference in the treatment group versus the placebo group in kcal/mile, kcals and miles show that Spirulina supplementation may decrease physical fatigue. This is consistent with the findings of two previous studies which note a longer time to exhaustion with participants taking the Spirulina supplement (5, 8). Another point of interest may be the effect that Spirulina had on the male subgroup in the present study. The significant difference in the males in the treatment group compared to the males in the placebo group show that the effect of Spirulina on physical fatigue may be
more potent in males than females. However, another possibility for this finding is that the male treatment group had a higher starting potential. This group started with a higher rate (kcals/mile) from baseline. This could influence the data as the male placebo group and the male treatment group did not evenly match up in terms of innate ability.

The data looking at the improvement in physical fatigue measurements over the course of the study did not show any significance for the Spirulina or placebo groups. Over the course of the approximate 8 weeks of Spirulina supplementation there was no statistically significant difference in the physical results at baseline compared to any of the various time points of the study. A previous study mentioned had a longer period of exercise to address physical fatigue (2 hours) (5). It is possible that we would have seen a greater change over time in the Spirulina group if a longer physical fatigue measure would have been used. In addition, the small sample size of 17 and 20 individuals within a group could limit the ability to find statistical significance. Future studies conducted could include a higher sample size. Also it would be ideal if the variability among the groups innate ability at baseline was as similar as possible.
**Mental Fatigue:**

The statistically significant change in scores over time from baseline to last day of supplementation was only observed in the treatment group. This may indicate a decrease in mental fatigue and increase in mental agility when given Spirulina. However, there was not a statistically significant difference in scores between the Spirulina and placebo groups. Notably, there was higher baseline ability in the placebo group than in the treatment which may affect the results of the comparison. Further research could include a study with a higher amount of study participants to see if there was any significant difference between the groups. Another interesting factor would be to increase the dose of Spirulina per day and see if there were any additional effects.

**Multidimensional fatigue test:**

The results from the T tests showed that the only significance was in the male treatment group compared to the male placebo group. This statistically significant difference in scores between these two groups indicates the possible effect of Spirulina on fatigue especially in the male subgroup. However, this significance was only seen on a one tailed T test which limits its
statistical validity. In addition, there was no significant change over time in the Spirulina group as well as no significant difference in scores between the placebo and the treatment group. The lack of change in these data was consistent with the N-of-1 fatigue study which showed no significant difference in fatigue scale measurements between the treatment and the placebo (7). A possible issue with this measure of fatigue was the placebo effect as this was a subjective self reported questionnaire on daily fatigue.

**C Reactive Protein:**

There was no significant difference between the CRP levels of the treatment and placebo groups. In addition, there was no significant change seen over time in the treatment group. Females in the treatment group had a non significant reduction in CRP levels by 0.4. CRP is just one blood marker measuring inflammation. Additional studies on these blood samples using different inflammatory markers may demonstrate some more information. In addition, the small sample sizes could cause difficulty in finding a significant difference.
Chapter 6: Conclusion

Two major points were analyzed in this randomized double blind clinical trial: fatigue and inflammation. These measures were addressed by analyzing physical fatigue, mental fatigue, a general fatigue scale, and CRP from blood specimens. Spirulina may decrease physical fatigue as seen in the difference between the placebo and the treatment group. In addition, Spirulina may have an effect in decreasing mental fatigue and increasing mental performance especially when used over time. In this study, Spirulina did not have a significant effect on CRP blood markers. Other inflammatory blood markers would be useful in identifying any changes not seen on the CRP analysis. Further clinical trials with higher sample size as well as studies addressing the dose response of Spirulina would be invaluable to continue to learn about its effects.
References:


