Effect of *Spirulina maxima* Supplementation and a Systematic Physical Exercise Program on Body Composition and Cardiorespiratory Fitness of Overweight or Obese Subjects: A Double Blind Randomized, Crossover Controlled Trial

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Abstract

Background: Overweight and obesity are major risk factors for many chronic diseases, and weight-loss interventions often include systematic exercising and nutritional supplements. The purpose of this study was to determine the independent/synergistic effects of *Spirulina maxima* supplementation (6-week, 4.5g/day) and a systematic physical exercise program (6 weeks/ 2 times) on body composition and cardiorespiratory fitness of overweight and obese subjects. Methods: 27 overweight and 25 obese sedentary male subjects were assigned to four interventions through a randomized double-blind, crossover controlled trial: Physical exercise program with (SE) or without (Ex) *Spirulina maxima* or no-exercise program with (Sm) and without (C) *Spirulina maxima*. Body composition and cardiorespiratory fitness parameters were taken during a maximum intensity test. Results: As compared to C group, SE, Sm and Ex groups reduced (p<0.05) their body fat percentage while improving their maximal oxygen uptake (r= -0.40), being obese subjects more benefited. Weight loss, time to reach fatigue and onset of blood lactate accumulation were improved in both *Spirulina maxima* supplemented groups, regardless the subjects’ body composition. Conclusions: *Spirulina maxima* supplementation synergistically improves the effects of systematic exercise in body composition and cardiorespiratory fitness parameters in overweight but mostly in obese adults.

Key words: Overweight; obesity; body fat; maximal oxygen uptake; double-blind; randomized controlled trial.


1. Introduction

Overweight and obesity [body mass index (BMI)≥ 25 kg·m⁻²] are major risk factors for many non-communicable chronic diseases including type-2 diabetes, cardiovascular diseases (CVD) and...
cancer [1]. Behavioral-based interventions focused on promoting a healthy diet and physical activity, have proven to be effective in reducing people’s body weight (BW) and body fat stores [2]. Systematic exercise practice improves people’s general fitness [e.g. higher maximal oxygen uptake (VO₂ max)], a prolonged onset of blood lactate accumulation (OBLA), a better ventilatory anaerobic threshold, and overall improvement of resting heart rate (RHR) and body fat mobilization [3,4]. However, behavioral-based interventions are even more effective when combined with prescribed drugs or nutritional supplements aimed to reduce fat stores and the body’s energy expenditure [5,6]. Marine product-based supplements are important sources of bioactive compounds [7], in this sense, *Spirulina sp.*, a group of cyanobacteria cultivated around the world, is a marine nutritional/nutraceutical supplement due to its content of phytochemicals (phenolic compounds, carotenoids and tocopherols) and essential nutrients (proteins, n-3 and n-6 fatty acids) [8]. Recently, studies reporting the use of *Spirulina maxima* (*S. maxima*) have been focused on demonstrating the biological activity of its components including but not restricted to its antioxidant and hypolipidemic activity [9,10]. However, most studies have been conducted in animal models, with scarce studies focused on its effects in humans [11,12] and no controlled studies focused on the effect of the administration of *S. maxima* and a systematic exercise program in humans have been reported yet [13]. Therefore, the goal of this study was to evaluate the independent and synergistic effect of *S. maxima* supplementation with or without a systematic physical exercise program on body composition [BW and body fat percentage (%BF)] and cardiorespiratory fitness [VO₂ max, anaerobic threshold, RHR, OBLA and time to fatigue (TF)] parameters. The differential effects in overweight and obese male adults are also reported.

2. Results

Baseline measurements of study participants (27 overweight and 25 obese) are shown on Table 1, while results in body composition and cardiorespiratory fitness of all four intervention groups [Physical exercise program with (SE) or without (Ex) *S. maxima* or no-exercise program with (Sm) and without (C) *S. maxima*] are depicted in Figures 1-4.

| Table 1. Anthropometric and physiological characteristics of participants. |
|--------------------------|----------|----------|----------|
|                         | Total    | Overweight| Obesity  |
| N                       | 52       | 27        | 25       |
| Age (y)                 | 26±5     | 26±4      | 27±6     |
| Body weight (kg)*       | 90±13    | 81±8      | 100±12   |
| Height (m)              | 1.72±0.1 | 1.72±0.1  | 1.73±0.1 |
| BMI (kg·m⁻²)*           | 30.2±4   | 27.4±1.2  | 33.3±3.8 |
| Body fat percentage*    | 28.8±7.2 | 24.8±5.9  | 33.2±6.1 |
| Energy intake (kcal·day⁻¹)| 2,054±104| 1,977±139 | 2,054±151|
Maximal Respiratory exchange ratio

|                | 1.19±0 | 1.19±0 | 1.18±0.1 |

Resting HR (bpm)

|                | 64.4±9.3 | 63.8±6.9 | 65.2±11.4 |

%HR at OBLA

|                | 61.9±11.8 | 62.9±11.5 | 60.8±12.2 |

VO\textsubscript{max} (mL•kg\textsuperscript{-1}•min\textsuperscript{-1})

|                | 35.4±6.9 | 39.6±5.1 | 30.8±5.6 |

Data is expressed as mean±SD. Asterisk means statistical differences between overweight and obese individuals (p<0.05); N= Sample size, BMI= Body mass index, HR= Heart rate, OBLA= Onset blood lactate accumulation, VO\textsubscript{max}= Maximal oxygen uptake.

Individual differences are often of wide range, for that reason the results are shown as differences between initial evaluations and after treatments. Also, since one individual (either performing or not the exercise protocol) underwent a crossover trial with or without S. maxima, all changes depicted in Figures 1-4 were corrected for basal BW and BMI.

2.1. Diet

Daily energy intake during the six weeks supplementation showed no statistical significant differences (p<0.05) between treatments for dietary variables at the beginning (2054±104 kcal•day\textsuperscript{-1}) compared with the final of the study (2146±98 kcal•day\textsuperscript{-1}). No dietary or S. maxima supplementation adverse effects were reported during the study.

2.2. Body Composition

Initially, obese individuals had a higher BMI (5.9 kg•m\textsuperscript{-2}) and %BF (8.4%) than overweight individuals (Table 1). According to Figure 1A, BW changes were more evident (p<0.05) in S. maxima-supplemented groups [-2.2 kg (SE) and -1.6 kg (Sm)] than that observed for non-supplemented ones [-0.8 kg (Ex) and -0.3 kg (C)], but obese subjects (Figure 1C) were more benefited than those overweight (Figure 1B). SE, Sm and Ex groups also reduced (p<0.05) their %BF (Figure 1D), regardless the subjects’ BW status. Lastly, according to Table 2, body weight change (ΔBW) was statistically (p<0.05) related to changes in body fat (r= 0.3) and RHR (r= 0.3), and to TF (r= 0.5) and VO\textsubscript{max} (r= -0.5) during the maximal intensity test (MIT).
Figure 1. Changes in body weight and body fat percentage by treatments. SE: *Spirulina* and exercise; Ex: Exercise and placebo; Sm: *Spirulina* without exercise; C: Control (Placebo treatment). A) Body weight changes in total of subjects B) Body weight changes in overweight subjects C) Body weight changes in obesity subjects D) Body fat changes in total of subjects E) Body fat changes in overweight subjects F) Body fat changes in obesity subjects. Data presented like mean ± SEM. Different letters indicate statistical difference between groups (p<0.05).

Table 2. Correlation matrix among physical and physiological variables.

<table>
<thead>
<tr>
<th></th>
<th>Δ BW</th>
<th>Δ %BF</th>
<th>Δ VO₂max</th>
<th>Δ OBLA</th>
<th>Δ RHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ %BF</td>
<td>0.33**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ VO₂max</td>
<td>-0.54**</td>
<td>-0.40**</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ OBLA</td>
<td>-0.17</td>
<td>-0.14</td>
<td>0.11</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Δ RHR</td>
<td>0.34**</td>
<td>0.06</td>
<td>-0.22*</td>
<td>-0.04</td>
<td>1</td>
</tr>
<tr>
<td>Δ TF</td>
<td>-0.50**</td>
<td>-0.19</td>
<td>0.36**</td>
<td>0.16</td>
<td>-0.38**</td>
</tr>
</tbody>
</table>

Δ= differences between before and after the study, BW= Body weight, %BF= percentage of body fat, VO₂max= Maximal oxygen uptake, OBLA= Onset blood lactate accumulation, RHR= Resting heart rate, TF= Time to fatigue. * p<0.05, ** p<0.01

2.3. Cardiorespiratory Fitness
The maximal oxygen uptake (VO₂max) in overweight subjects was 8.8 ml.kg⁻¹.min⁻¹ more than that of obese individuals but resting HR in both groups was not different (Table 1). Changes in TF by treatments (Figure 2) mainly favored obese subjects (Figure 2C); RHR (Figure 3A) and OBLA (Figure 3D) both with no apparent effect of BMI status were mostly improved in *S. maxima* supplemented groups (SE and Sm).
Figure 2. Changes in time to fatigue by treatments. SE: *Spirulina* and exercise; Ex: Exercise and placebo; Sm: *Spirulina* without exercise; C: Control (Placebo treatment). A) Time to fatigue changes in total of subjects B) Time to fatigue changes in overweight subjects C) Time to fatigue changes in obesity subjects. Data presented like mean ± SEM. Different letters indicate statistical difference between groups (p<0.05).

Figure 3. Changes in resting heart rate and onset blood lactate accumulation by treatments. HR: Heart rate; OBLA: Onset blood lactate accumulation; %HR: Percentage of maximal heart rate SE: *Spirulina* and exercise; Ex: Exercise and placebo; Sm: *Spirulina* without exercise; C: Control (Placebo treatment). A) Resting HR changes in total of subjects B) Resting HR changes in overweight subjects C) Resting HR changes in obesity subjects D) OBLA changes in total of subjects E) OBLA changes in overweight subjects F) OBLA changes in obesity subjects. Data presented like mean ± SEM. Different letters indicate statistical difference between groups (p<0.05).

Also, VO$_{\text{max}}$ improved in SE, Sm and Ex groups as compared to C group (Figure 4A), being obese individuals (Figure 4C) more benefited than overweight ones (Figure 4B). According to Table 2, besides correlating with Δ%BW, ΔVO$_{\text{max}}$ also correlated (r) with Δ%BF (-0.4), ΔTF (0.4) and ΔRHR (-0.2).
Figure 4. Changes in maximal oxygen uptake by treatments. VO2max: maximal oxygen uptake. SE: Spirulina and exercise; Ex: Exercise and placebo; Sm: Spirulina without exercise; C: Control (Placebo treatment). A) VO2max changes in total of subjects B) VO2max changes in overweight subjects C) VO2max changes in obesity subjects. Data presented like mean ± SEM. Different letters indicate statistical difference between groups (p<0.05).

3. Discussion

This study is the first focused on examining the effects of S. maxima supplementation in body composition in humans using a double-blind, randomized, crossover trial design. The main findings of this study about the effects of S. maxima supplementation (4.5 g) alone or together with the systematic practice of exercise on body composition were a significant decrease in BW and %BF, during six weeks of treatment. About the cardiorespiratory parameters it was found that there is a significant improvement in RHR, TF, VO2max, and %HR at OBLA according to the S. maxima supplementation and/or systematic practice of exercise.

3.1. Body Composition

The study of the potential effects of nutraceutical supplements is increasing. Miczke et al [14], reported the effect of 2 g•day⁻¹ of S. maxima supplementation on 40 hypertension subjects in a double-blind, placebo-controlled randomized trial for three months. After the intervention, they reported a significant reduction of BW, BMI and blood pressure in the group supplemented with S. maxima compared with that of the placebo group. Mazokopakis et al [15], studied the effect of Spirulina supplementation (1 g•day⁻¹) during three months in BW, BMI, and lipid profile in patients with dyslipidemia and reported a significant decrease in total cholesterol, triacylglycerols, and cholesterol associated to low-density lipoproteins. In our study, we found a significant decrease on BW, and %BF in all treatments compared with that of the control; even though there was difference between treatments in both groups, there was a tendency to an additive effect of S. maxima and exercise. The inconsistent results in the Ex group was probably due there exist a considerable interindividual variability in the magnitude of weight loss produced by exercise and there doesn’t exist a specific kind of exercise program to overweight and obese persons [16]. The S. maxima groups showed better results about body composition probably due their specific action mechanism in the organism, Fujimoto et al [17], hypothesized that some Spirulina compounds could reduce the macrophages infiltration into visceral fat and prevents liver lipids accumulation, resulting in weight reduction, specifically body fat, what would explain our clinical trial results, but there are necessary studies focused in elucidate the specific compounds and action mechanisms by which Spirulina affects body composition.
3.2. Cardiorespiratory Fitness

3.2.1. Time to Fatigue

The effects granted by *S. maxima* observed in the present study strengthen the data reported by Kalafati *et al* [12], who studied the effect of *S. maxima* supplementation (6 g•day⁻¹) in nine recreational runners training at least two days a week, 45 min per session, obtaining a significant improvement in TF in a submaximal exercise test (~40 s) after 4 weeks of the clinical trial. In addition to the VO₂max increase observed here, it is probable that some of the compounds present in *S. maxima* like phycocyanins, essential fatty acids, carotenoids or phenolic compounds act individually in different metabolic processes to improve cardiopulmonary fitness; these effects are discussed in the next sections.

3.2.2. Resting Heart Rate and OBLA

A high RHR has been associated with cardiovascular mortality in numerous epidemiologic studies [18,19]. Torres-Duran *et al* [20], studied the effect of *S. maxima* supplementation (5 g•day⁻¹) during 15 days in lactate concentration of recreational runners (n=41) before and after an intensive exercise. Their results showed a trend to lower lactate concentration (mM) before exercise with *S. maxima* supplementation (1.7±0.6) compared with exercise without supplementation (2.3±2.2); and, the results were similar in lactate concentrations after exercise with *S. maxima* supplementation (12.8±6.3) compared with exercise without supplementation (15.5±4.2). Moreover, they did not quantify the lactate concentrations through the exercise test, for that reason it was not possible to determine the OBLA, but our results in the decrease of RHR can be directly related only with the increase of the VO₂max in each MIT; and, even though the trial duration was of short term, we found minimum but statistical differences after treatments. Lu *et al* [21], examined the effect of *Spirulina* supplementation (7.5 g•day⁻¹) in lactate concentrations before maximal athletic activity in a randomized double-blind placebo-controlled study in 16 young students. They reported a statistical decrease (p<0.05) in blood lactate concentration (mM) after supplementation (three weeks) compared with the control before performing a maximal athletic activity (2.3±0.3 vs 3±0.4). This study strengthens our results that a high protein content nutraceutical like *Spirulina* can improve some biochemical parameters like RHR and blood lactate concentration, which can be the starting point to focus studies in these response variables in different kinds of populations. Our results in RHR and %HR at OBLA may be due *S. maxima* proteins exert an anti-inflammatory role or indirectly modulate the inflammatory state and the balance of muscle cells in order to favor the biological response and the adaptation of the muscle tissue during the MIT; the possible action mechanism may involve the suppression of skeletal muscle proteolysis and the stimulation of protein synthesis due the high quality proteins content in *S. maxima* [22]. Regarding OBLA, conclusions of different researches suggest the utilization of anaerobic exercise sessions with high-intensity interval training (HIIT) to induce blood lactate accumulation provides stimulus to improve aerobic performance markers such as OBLA [23]; our OBLA results could not have correlation with any of the other cardiorespiratory parameters analyzed due the physiological adaptations in sedentary overweight and obese subjects to exercise could be lower than in active population [24].

3.2.3. Maximal Oxygen Uptake

In our study, there was a low but statistical increase in VO₂max in SE and Sm groups; this increase may not be of clinical significance since it is due to the body mass loss rather than to an
improvement in cardiorespiratory fitness. Similar to our results, De Strijcker et al [25], reported an intervention with 10 weeks of HIIT (twice a week) in 16 male overweight/obese subjects. They showed a low but statistical increase in VO2max (~0.2 ml•min⁻¹•kg⁻¹) and a decrease in BW (~1 kg). According to this, a growing body of evidence suggests that regular exercise training improves health benefits like VO2max and body mass, but the optimal intensity and volume necessary to obtain maximal benefits remains to be defined [26]. The VO2 improvements can be affected by both aerobic capacity and the intensity of exercise [27], and even in our study the training intensity of sedentary subjects was according to the one recommended by the American College of Sport Medicine (ACSM) to improve cardiorespiratory fitness (>40% of VO2max) [24]. The ideal length and duration of studies in sedentary adults are not well defined, showing clinical effects from 20 min•day⁻¹ during five weeks at 55% of VO2max to 36 min•day⁻¹ during 24 weeks at 50% of VO2max [28]. For that reason, we considered that using a training program at a moderated and high intensity of 30 min•day⁻¹ for 12 weeks was appropriate to the improvement of the cardiorespiratory parameters of our study population.

The correlation between body composition and VO2max could be due the exercise program effect was granted by the cyanobacteria due to: 1) Spirulina regulates lipolysis due to the adipose tissue effect showed probably by action of its fatty acids composition[29]: 2) The proteins content in S. maxima (~65%) inhibits the gut lipid absorption[30], resulting in mild steatorrhea [31], but the research has focused mainly in animal models, so studies are required to prove this is similar in humans.

Our positive results in S. maxima intake groups in the cardiorespiratory parameters suggest a high bioavailability of antioxidant compounds present in the cyanobacteria could have a synergistic effect against oxidative stress caused by the MIT; carotenoids present in S. maxima could be exceptional quenchers of reactive oxygen species induced by extenuate physical activity, inhibiting lipid peroxidation [32], resulting in an improve of cardiorespiratory performance at major utilization of lipids like energy substrates. This indicates that focusing clinical studies on the intake of S. maxima, could result in positive effects not only in body composition and cardiorespiratory fitness, but also in diseases related to oxidative stress.

Even though the present study shows evidence of clinical importance of S. maxima supplementation and exercise, there is a limitation to determinate the correct action mechanisms due to the variety of compounds present in S. maxima [33]. Further studies are necessary to elucidate the S. maxima action mechanisms and to determine the optimal intake for humans to improve general fitness; among them, increase the sample, the duration of the treatments, and study different populations of both sexes.

4. Materials and Methods

Spirulina maxima was obtained commercially from Alimentos Esenciales para la Humanidad S.A. de C.V. (Mexico City, Mexico) on January 2017, and was characterized (Supplementary File 1) before the clinical trial.

The Consolidated Standards of Reporting Trials (CONSORT) Checklist (Supplementary File 2) and the CONSORT Flow Diagram (Supplementary File 3) of the progress of the phases through the trial are available as supporting information. A detailed protocol for this trial has been previously
published [34]. All participants were informed of study purposes, physical, clinical and biochemical procedures, also about the physical intensity tests’ risks. Their acceptance was formalized by means of informed consent and their confidentiality was strictly enforced. The Universidad Autónoma de Ciudad Juárez (UACJ) Review Board approved the study and all procedures (Reference number: CBE.ICB/062.09-15); the trial registration number at clinicaltrials.gov is NCT02837666 (Hypolipidemic and Antioxidant Capacity of Spirulina and Exercise). This study was conducted in accordance with the Declaration of Helsinki.

4.1. Participants’ Eligibility

Fifty-two sedentary [daily energy expenditure <4 metabolic equivalents (METs) as measured by a continuous physical activity questionnaire (IPAQ)] male adults with BMI over 25 kg•m⁻² volunteered to participate from May to September, 2017. The exclusion criteria of subjects were drinking more than 100 mL of alcohol a week, taking drugs and/or diet supplements, present a chronic disease, or an impediment to practice regular physical exercise.

4.2. Baseline Measurements

At the beginning of the study subjects visited the exercise physiology laboratory at UACJ for baseline measurements. Body measurements were performed with subjects lightly dressed and barefoot using an electronic balance while standing height was measured with a stadiometer. Participant’s RHR was measured after five min of lying down in a bed. %BF was measured by plethysmography (BodPod, COSMED, Rome, Italy) according to the manufacturers’ guidelines. All measurements were repeated before each MIT.

4.3. Study Design

The clinical trial consisted of *S. maxima* intake during 12 weeks in a randomized, double-blind, placebo-controlled, counterbalanced, crossover design aimed to eliminate inter-individual bias as well as boosting the reliability of results. 52 eligible participants were assigned to four interventions through a randomized double-blind, crossover controlled trial: Physical exercise with (SE) or without (Ex) *S. maxima* or no-exercise (sedentary) with (Sm) and without (C) *S. maxima* (Figure 5). Initial allocation was performed in such way that each group had almost the same proportion of overweight/obese (1:1) individuals using a computer-generated random schedule stratified by site and the baseline score of the Action Arm Research Test using permuted blocks of random sizes, receiving either 4.5 g placebo (low calorie saccharine powder) or *S. maxima* daily, in a non-transparent capsule (to mask appearance and taste) with or without exercise, 2×2 crossover. The first period of treatment was carried out in six weeks, followed by a two weeks wash out period and finally six weeks for the second intervention groups (Figure 5). Participants’ group allocation was performed by an independent researcher who did not have any other participation during the study (double-blind). The sample size was determined by using the statistical program G*Power [35], selecting a sample of minimum 52 subjects with α=0.05 and power=0.85.
Figure 5. Experimental design for independent and additive effect of *Spirulina maxima* and exercise. MIT: Maximum intensity test.

### 4.4 Maximum Intensity Test (MIT)

Each subject participated in four stress tests performed at maximum intensity during the study; during MIT, consumed O₂ and produced CO₂ were taken by a gas analyzer (Cortex MetaLyzer® 3B, Germany), HR with a Polar H7 sensor (Polar Electro, Lake Success, NY). The anaerobic threshold was determined when OBLA reached 4 mM. The MIT protocol consisted of using a cycle ergometer (Monark ergomedic 828 E; Monark exercise AB, 105 Vansbro, Sweden) initiating with a workload of 50-75 W with increments of 15-30 W every 3 min for a minimum of 9 min and until 15 min. At the end of each increment load, capillary blood samples were taken to determine lactate using a YSI 1500 Sport Lactate Analyzer (YSI Life sciences, OH, USA). HR was recorded, and the physical perceived effort was registered by using the Borg CR-10 scale. The anaerobic threshold was recorded through %HR at OBLA. According to the ACSM guidelines [24], the MIT was considered maximal if at least three of the following judgments were reached: failure of HR to increase with increases in workload, a peak respiratory exchange ratio ≥ 1.1, blood lactate concentration >8 mM, VO₂ plateau [failure of VO₂ to increase (150 mL•min⁻¹) despite increasing working rate], rating of perceived exertion at peak exercise>7.

The first MIT was performed before beginning the first treatment, the day after the first supplementation period, subjects returned to perform the second MIT with identical conditions to the first one. The third MIT was performed after a washout period of two weeks to remove the effects of treatment and avoid any possible delayed effect of *S. maxima* in the organism. The last MIT was performed after the second treatment with identical conditions to the previous three times.

### 4.5. Dietary Analysis

All participants were subjected to a nutritional survey to define the daily calories required to establish dietary recommendations. Dietary intake was monitored through retrospective methods.
including the 24 h recall (which investigates intake over a specific day), and the food frequency 
questionnaire (a summary of usual intake of different categories of foods). Dietary intake 
evaluations were performed at each MIT of the study to record the possible variability in food 
consumption patterns. The diet records were analyzed for total calories, protein, carbohydrate, and 
fat intake (Diet Analysis Plus, ESHA Research, Salem, OR).

4.6. Physical Exercise Protocol
SE and Ex participants exercised five days a week with the following protocol: between 5 and 10 min 
of warm-up exercise, between 20-30 min muscular endurance exercise and 20-30 minutes of aerobic 
exercise (cardiovascular exercise): walking, jogging, running and/or cycling, and five final minutes 
of stretching. The cardiovascular exercise was divided into three days a week, aerobic intensity 
between 50% and 80% of maximal HR reserve, and two days between 80% and 90% using HIIT.

4.7. Main Physiological Outcomes
The primary outcome of the study was the body composition response because the excess body fat is 
one of the major risk factor to CVD and health organizations around the world recommend studies 
focused in this response variable [1].
We included like secondary endpoints the cardiorespiratory fitness variables, including TF, RHR, 
%HR at OBLA and VO2max; due they have been associated to cardiovascular mortality in numerous 
epidemiologic studies, regardless of whether people presented any CVD [36].

4.8. Statistical Analysis
Data distribution normality was examined by the Shapiro-Wilk test on each response variable. In 
order to analyze differences between overweight and obesity subjects, a Mann-Whitney U test was 
performed; and to analyze differences between treatments before and after the study, the Δ were 
analyzed by Kruskal-Wallis H and Mann-Whitney U tests. To evaluate the association among 
variables a Spearman correlation was performed. Statistical significance was considered when 
p<0.05, using the software SPSS 22.0 (SPSS Inc., Chicago, IL) in all analyses.

5. Conclusions
Spirulina maxima intake together a systematic physical exercise program, have an individual and 
synergistic beneficial effect on body composition (decrease of BW and %BF) and cardiorespiratory 
parameters (increase of TF, VO2max, OBLA, and reduction of RHR) in overweight but mostly in 
obese male adults.

Supplementary Materials: The following are available online, Supplementary File 1: Partial chemical 
characterization of Spirulina maxima, Supplementary File 2: CONSORT 2010 Checklist of information to include 
when reporting a randomized trial, Supplementary File 3: CONSORT 2010 Flow Diagram of the progress 
through the phases of the trial.

Author Contributions: First author, MAHL was commissioned to the participants’ recruitment and led the 
development of this manuscript. ARJ and MAJO designed the study procedures and perform the statistical 
analyses of the data. RPHT conducted the physical exercise program. AWL and JALD designed the diet 
individual dietary recommendations to all the participants of the study. All authors made critical revisions to 
the manuscript a number of times and approved the final version.

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