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Fourteen-Days Spirulina Supplementation Increases Hemoglobin, but Does Not Provide Ergogenic Benefit in Recreationally Active Cyclists: A Double-Blinded Randomized Crossover Trial

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ABSTRACT

Spirulina supplementation has been reported to increase hemoglobin concentration as well as a variety of cardiorespiratory and lactate-based performance parameters during maximal and submaximal states of exercise. This study investigates the efficacy of supplementing a 6 g/day dosage of spirulina for 14-days in recreationally active individuals, analyzing cardiorespiratory parameters during maximal and submaximal cycling as well as the potential mechanistic role of hemoglobin augmentation. 17 recreationally active individuals (Male = 14, Female = 3, Age 23 ± 5 years, VO_{2max} 43.3 ± 8.6 ml/min·kg) ingested 6 g/day of spirulina or placebo for 14-days in a double-blinded randomized crossover study, with a 14-day washout period between trials. Participants completed a 20-min submaximal cycle at 40% maximal power output (WR_{max}), followed by a VO_{2max} test. Hemoglobin (g/L), WR_{max} (watts), time to fatigue (seconds), heart rate (bpm), oxygen uptake (ml/min·kg), RER and blood lactate response (mmol/L) were measured and compared between conditions. Cardiorespiratory variables were recorded at 5-min intervals and lactate was measured at 10-min intervals during the submaximal exercise. There was a significant 3.4% increase in hemoglobin concentration after spirulina supplementation in comparison to placebo (150.4 ± 9.5 g/L Vs 145.6 ± 9.4 g/L, $p = 0.047$). No significant differences existed between either condition in both testing protocols for $VO_{2max,}$ WR_{max}, time to fatigue, heart rate, oxygen uptake, RER and blood lactate response ($p > 0.05$). 14-days of spirulina supplementation significantly improved hemoglobin concentration but did not lead to any considerable ergogenic improvements during maximal or submaximal exercise at a 6 g/day dosage in recreationally active individuals whilst cycling.

Abbreviations: ANOVA: analysis of variance; cGMP: cyclic guanosine monophosphate; Hb: hemoglobin; HR: heart rate; RER: respiratory exchange ratio; RPM: revolutions per minute; TBARS: thiobarbituric acid reactive substances; TAC: total antioxidant capacity; TTF: time to fatigue; VO2: oxygen consumption; VO_{2max}: maximal oxygen consumption; WR_{max}: maximal power output

KEYWORDS
Algae; cycling; hemoglobin; spirulina

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Introduction

Spirulina, a cyanobacterium often described as blue-green algae, has been reaping interest due to the plethora of possible health benefits it offers (Grosshagauer et al. 2020). Commonly referred to as a 'superfood' (Dominguez 2013), spirulina's nutritional content is ample with micronutrients, vitamins, and minerals, boasting a dry mass of approximately 70% protein (Franca et al. 2010; Aouir et al. 2017).

Research has demonstrated the clinical versatility of spirulina's nutritional composition through a multitude of benefits, including positive effects against hyperlipidemia, malnutrition, obesity, diabetes, and anemia as well as antioxidant and anti-inflammatory activity (Mani et al. 2000; Lee et al. 2008; Selmi et al. 2011; Hosseini et al. 2013; Ichimura et al. 2013; Wu et al. 2016; Hernández-Lepe et al. 2018). Consequently, this has evoked an interest into spirulina and whether it can also perform as a potential ergogenic aid.

Current literature has reported spirulina supplementation to increase $\dot{V}O_{2max}$ in both cycling (Kalpana et al. 2017; Hernández-Lepe et al. 2018) and arm-cycling (Gurney and Spendiff 2020), as well as decreasing heart rate (HR) (Gurney and Spendiff 2020) and RER (Kalafati et al. 2010) whilst increasing the time taken to fatigue during running (Lu et al. 2006; Kalafati et al. 2010). Research has also elucidated spirulina to elicit improvements in hemoglobin (Hb) levels across both healthy (Gurney et al. 2021.; Kelkar et al. 2008; Milasius et al. 2009; Gurney and Spendiff 2020;) and clinical populations (Mani et al. 2000; Selmi et al. 2011). Increases in Hb have been suggested to augment performance due to its crucial role in oxygen transport (Mairbäurl 2013). Clinical and performance-based research have attributed increases in Hb levels to the high assimilation and bioavailability of iron present in spirulina (Gurney and Spendiff 2022). Hb consists of a protein component (globin) and an iron complex of a porphyrin derivative (heme), thereby making iron fundamental to the synthesis and structure of Hb (Dedhia 2000). However, in studies where cyclists with adequate nutritional status were investigated (Franca et al. 2010) or during resistance training – an exercise modality different to running and cycling (Pappas et al. 2021), no ergogenic benefits were reported.

The current mechanistic avenues explored have focused on the antioxidant properties of spirulina thought to occur due to its phycocyanin, β-carotene and cysteine content (Gurney and Spendiff 2022). Spirulina supplementation has been found to improve a variety of oxidative stress and lipid peroxidation biomarkers such as increasing glutathione levels and superoxide dismutase activity whilst decreasing malondialdehyde (MDA), total antioxidant capacity (TAC) and thiobarbituric acid-reactive substances (TBARS) (Lu et al. 2006; Kalafati et al. 2010; Wu et al. 2016). However, some of these measures, namely MDA, TBARS and TAC, have been reported to oversimplify the complex nature of redox active therapeutics. They are susceptible to methodological errors and are therefore no longer recommended for use as a metric of oxidative stress (Cobley et al. 2017). Very few studies have investigated the influence of spirulina supplementation on F2-isoprostanes, which is considered to be the gold standard for measuring oxidative stress (Morrow 2005). However, a recent study by Chaouachi et al. (2022) has done so, observing significant increases in F2-isoprostanes, C-reactive protein (CRP), and creatine kinase (CK) levels in the placebo group and not the spirulina group immediately after exhaustive exercise. Overall, these findings support previous literature whereby spirulina supplementation may prevent oxidative stress, lipid peroxidation, and inflammation (Chaouachi et al. 2022).

Nonetheless, spirulina's underlying mechanisms still remain equivocal, thus allowing scope for other possible hypotheses to be a reason for ergogenic benefits – specifically the high iron content that is thought to result in an increase in Hb (Gurney and Spendiff 2022).

Despite evidence of ergogenic improvements, the optimal dosage and duration of spirulina supplementation remains unclear. Supplementation periods have extended up to 60 days (Selmi et al. 2011), with daily dosages varying from 1.5 g to 7.5 g (Lu et al. 2006). Notably, 6 g/day has shown efficacious results across various intensities during running (Kalafati et al. 2010), cycling (Gurney et al. 2021), and arm-cycling (Gurney and Spendiff 2020), including 30- and 60-min bouts at 55% $\dot{V}O_{2max}$ (Gurney et al. 2021; Gurney and Spendiff 2020) and a 2-h bout at 70% $\dot{V}O_{2max}$ (Kalafati et al. 2010) in addition to maximal tests. The degree of efficacy of spirulina supplementation at varying submaximal intensities is yet to be ascertained, with scope to uncover a minimum threshold intensity (Gurney et al. 2021; Kalafati et al. 2010; Gurney and Spendiff 2020). Notably, a 40% intensity is yet to be assessed; the novelty of investigating lower exercise intensities could potentially reveal whether a minimum amount of exercise-induced stress is required for spirulina supplementation to provide any ergogenic benefit. Submaximal loads of this intensity, especially 40%, are applicable to a broader caliber of individuals ranging from recreationally active to elite, as it generally corresponds with Zone 2 HR training, which is often used to develop endurance and thus beneficial within a submaximal remit. Furthermore, individual performance at submaximal intensities for extended periods of time is pertinent in endurance sport, as a diminished capacity for submaximal exercise directly translates to a reduced level of stamina (Hinton 2014).

The value of a 6 g/day dosage still warrants further investigation due to the dearth of existing literature. This study will therefore examine a 6 g/day dosage, in alignment with previous research (Gurney et al. 2021; Kalafati et al. 2010; Gurney and Spendiff 2020) but will employ a 14-day supplementation protocol; to the best of our knowledge this timeframe has not yet been investigated whilst looking at exercise performance measures. Consequently, the novelty of this study will expand upon and refine the current body of literature on spirulina supplementation, by attempting to ascertain the optimal supplementation timeframe, at a dosage concordant with previous research in the acute setting.

Therefore, the primary aim of this study was to investigate whether 6 g/day spirulina supplementation over 14-days can elicit any ergogenic aid to the key physiological parameters associated with cycling during 40% submaximal and maximal intensities in recreationally active individuals, whilst paying close attention to the potential role of Hb. It was hypothesized that 6 g/day of Spirulina supplementation for 14-days would provide ergogenic aid in recreationally individuals in submaximal and maximal cycling.

Materials and methods

Participants

17 participants completed the study (Male = 14, Female = 3, Mean ± SD; Age 23 ± 5 years, Stature 178 ± 8 cm, Weight 74.9 ± 11.4 kg, $\dot{V}O_{2max}$ 43.3 ± 8.6 ml/min·kg). Initially, 26 participants were recruited, however 9 participants were excluded as they did not meet the inclusion criteria – none were lost due to side effects or intolerance of the supplement (see Figure 1 – CONSORT flow diagram). The inclusion criteria was as follows: participants aged 18–50, exercising 3–4 h per week and able to attend the laboratory on 4 separate occasions to complete the testing. Any participants with an injury/illness that inhibited their ability to perform the exercise testing were excluded, alongside those with a history of smoking or taking any blood-thinning or immunosuppressing medication. Alongside informed consent, Physical Activity Readiness Questionnaires were completed to screen for any contraindications to exercise prior to participation. All methods were followed using the PRESENT2020 checklist.

G-power calculations to achieve 80% power (alpha = 0.05) and detect a small effect size 'f' of 0.3 (in an ANOVA: repeated measure, within factors) between supplementation and submaximal cardiorespiratory variables, estimated a sample size of 17. The effect size chosen was based on the expectation of a small-medium experimental effect size (Gurney and Spendiff 2020).

Study design

A double-blinded, placebo-controlled randomized crossover protocol was implemented to investigate the influence of spirulina supplementation on Hb and cardiorespiratory variables. The study entailed a 20-min submaximal bout of cycling at 40% WR_{max}, followed by a 15-min rest period and then a $\dot{V}O_{2max}$ test to volitional exhaustion. Hb (g/L), heart rate (HR) (bpm), oxygen uptake ($\dot{V}O_2$) (ml/min·kg), WR_{max} (watts), time to fatigue (TtF) (seconds), respiratory exchange ratio (RER) and blood lactate response (mmol/L) were measured and compared between conditions.

Participants attended the laboratory (Institute of Sports, Exercise and Health, London), on 4 separate occasions. The first visit comprised a $\dot{V}O_{2max}$ test, which determined the resistance to be used for the subsequent submaximal protocols (Gurney et al. 2021; Kalafati et al. 2010; Gurney and Spendiff 2020). The second visit familiarized the participants with the format of the exercise protocol employed (20-min submaximal cycle followed by $\dot{V}O_{2max}$ test) before beginning supplementation. Participants were then counterbalanced and randomly allocated either spirulina (Indigo Herbs Limited – see Table 1 for nutritional composition) or placebo (microcrystalline cellulose) capsules, with instructions to ingest 6 g/day postprandially with 4 capsules each containing 0.5 g spirulina, following breakfast, lunch and dinner, (Gurney et al. 2021; Gurney and Spendiff 2020), thus making a total of 12 capsules. Participants were instructed to take supplementation for a total of 14-days.

Both spirulina and placebo powders were placed into identical opaque capsules to disguise identification. An independent academic member of staff coded the capsules to prevent bias amongst participants and researchers. There was a minimum of 28-days

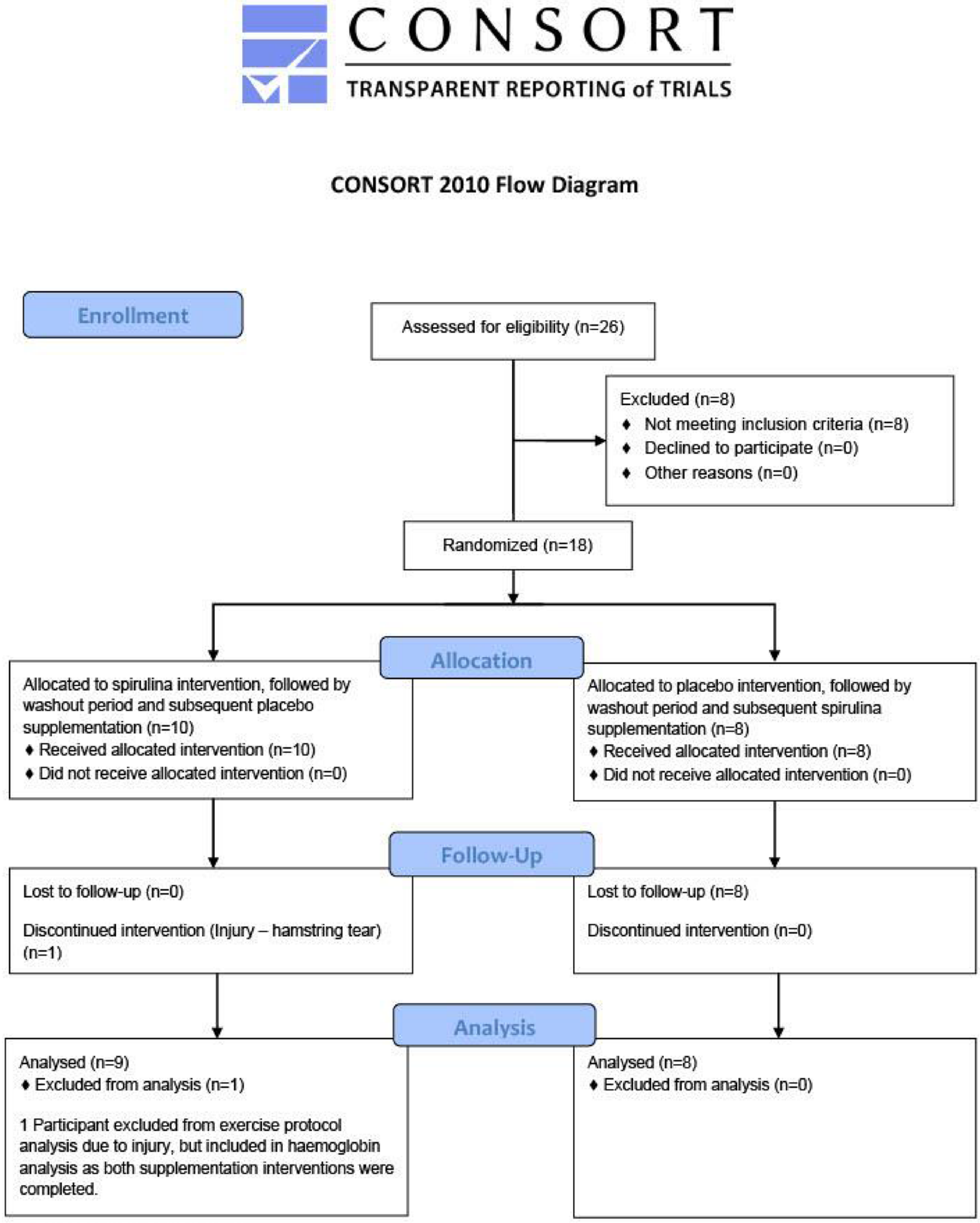

Figure 1. CONSORT flow diagram.

respite between the third and fourth visits to allow for a 14-day washout period (Gurney et al. 2021; Kalafati et al. 2010; Gurney and Spendiff 2020; Kashani et al. 2022) and a subsequent 14-day supplementation period (Figure 2).

Throughout the intervention, participants were instructed to maintain their regular training loads and intensities. Participants were also instructed to refrain from taking any additional supplementation and to not undergo any strenuous exercise 24-h before testing. Furthermore, 3-day activity and nutritional logs preceding each visit were

Table 1. Summarizing the nutritional composition of Spirulina given to participants.

Nutritional information	Per 100g	Per 6g daily dose	%RDA*	Total consumption over 14 days
Energy (KJ/Kcal)	1420KJ/339Kcal	85.2KJ/20.16Kcal	1.02%	1192KJ/285Kcal
Fat	1.00 g	0.06 g	0.85%	0.84 g
of which saturates	0.50 g	0.012 g	0.05%	0.017 g
Carbohydrate	13.10 g	0.79 g	0.80%	11.00 g
of which sugars	0.00 g	0.00 g	0.00%	0 g
Protein	65.90 g	3.95 g	7.91%	55.36 g
Dietary Fiber	6.40 g	0.38 g	0.19%	5.38 g
Salt	900.00 mg	54.00 mg	0.90%	756.00 mg
Vitamin A	0.34 mg	0.020 mg	2.57%	0.020 mg
Vitamin E	5.00 mg	0.30 mg	2.50%	4.20 g
Vitamin K	0.03 mg	0.0018 mg	2.00%	0.025 mg
Vitamin B1	2.40 mg	0.14 mg	13.09%	2.02 mg
Vitamin B2	3.70 mg	0.22 mg	18.86%	3.11 mg
Vitamin B3	12.80 mg	0.77 mg	4.80%	10.75 mg
Vitamin B6	1.40 mg	0.085 mg	6.00%	1.19 mg
Folate	0.09 mg	0.0050 mg	2.82%	0.07 mg
Pantothenic acid	3.50 mg	0.21 mg	3.50%	2.94 mg
Potassium	1363.00 mg	81.78 mg	4.09%	1144.92 mg
Calcium	332.50 mg	19.95 mg	2.50%	279.30 mg
Phosphorus	118.00 mg	7.08 mg	1.01%	99.12 mg
Magnesium	300.00 mg	18.00 mg	4.80%	252.00 mg
Iron	6.58 mg	0.39 mg	2.82%	5.53 mg
Zinc	2.00 mg	0.12 mg	1.20%	1.68 mg
Copper	6.10 mg	0.37 mg	36.60%	5.11 mg
Manganese	1.90 mg	0.16 mg	5.70%	2.17 mg
Selenium	0.01 mg	0.00 mg	0.79%	0.00 mg

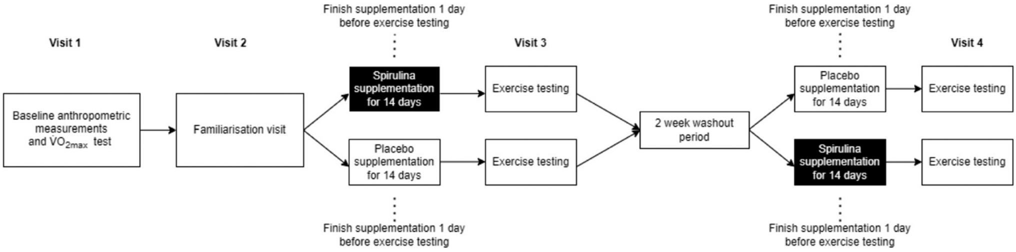

Figure 2. Schematic diagram of study design.

requested and asked to be replicated for the subsequent visits to minimize any confounding factors. Adherence to supplementation intake was ensured *via* weekly check-ins. An exit questionnaire was conducted to further assess adherence, efficacy of blinding and to understand if any issues were encountered with supplementation, e.g. nausea or gastrointestinal issues. Adherence was 100% and no gastrointestinal issues were reported.

Baseline measurements & VO_{2max} (V1)

Anthropometric measurements of body mass (kg) and stature (cm) were recorded during the first visit and at the beginning of each subsequent visit, using the Tanita (Tania Corp., Tokyo, Japan) and the Floor Stadiometer (Holtain Ltd., Dyfed, Wales), respectively. The saddle height of the bike and mask was adjusted to the participant's preference and kept consistent for subsequent visits, as was the time of visit relative to the participant. Hb levels were measured at the start of each visit *via* capillary finger-pricks using the HemoCue Hb201+ (HemoCue AB, Ängelholm, Sweden). Cardiorespiratory variables were measured using the Vyntus CPX (Vynaire Medical GmbH, Germany) and the Polar F10 strap (Polar Electro Oy, Kempele, Finland).

The $\dot{V}O_{2max}$ test was conducted on a Lode Cycle-Ergometer (Lode BV, Groningen, Netherlands). The test comprised a 2-min warmup at 75 watts and thereafter linearly increased by 25 watts every minute until the participants were unable to maintain a cadence of 60 revolutions per minute (RPM) for more than 10-s. Throughout testing, participants were asked to maintain an RPM between 60–80 (Gurney and Spendiff 2020).

The respiratory variables were analyzed and averaged every 15-s. $\dot{V}O_{2max}$ was determined by the highest $\dot{V}O_2$ value that was recorded from the 15-s averages before test termination (Gurney and Spendiff 2020). WR_{max} was taken as the peak value at the point of test termination and was recorded to subsequently establish each participant's 40% relative intensity for the 20-min submaximal exercise sessions.

Submaximal & maximal test (V2,3,4)

The corresponding resistance for each participant's 40% relative intensity work rate was applied to the ergometer for the 20-min submaximal cycle. $\dot{V}O_2$ and HR during the submaximal exercise tests were recorded and averaged to 60-s. Each 5-min interval was then calculated and averaged for analysis. Following the submaximal exercise, participants were given a 15-min rest period where water was provided ad libitum and the amount consumed was recorded and standardized for subsequent visits. This was followed by a $\dot{V}O_{2max}$ test, which followed the same protocol as per visit 1. Blood lactate was measured *via* capillary finger picks (Lactate Pro-2-ArkrayFactoryInc, 1480, Shiga, Japan) at 0, 10, 20 min during submaximal testing, two measurements were also taken at the end of the 15-min rest period and at the end of the $\dot{V}O_{2max}$ test. Test re-test reliability was conducted for the exercise protocol, using data collected external to the participants in the study, with all variables were expressed as CV% (see Supplementary File 1).

Statistical analysis

Statistical analysis was carried out using IBM SPSS version 28.0.0.0 for windows. Data and results are presented as mean ± SD. Data was analyzed for normality using the Shapiro-Wilk test. Hb, $\dot{V}O_{2max}$, time to fatigue and peak power output were compared using a two-tailed paired sample T-Test. To evaluate any differences throughout the submaximal protocols, VO_2, HR, RER were analyzed using a two-way within-subjects repeated measures ANOVA with a Bonferroni correction for multiple comparisons. Mauchly's Test of Sphericity was employed to establish any potential violations to the assumptions and any infringements were corrected using values from the Greenhouse–Geisser. Post-hoc paired-sample T-tests were used to evaluate any statistically significant differences in the data where significant main effects were found. Lactate clearance and response also utilized the same protocol of ANOVA testing.

Results

Hemoglobin

There was a small but significant increase in Hb following spirulina supplementation compared to placebo (150.4 ± 9.5 vs 145.6 ± 9.4 g/L, $p = 0.047$, ES with Hedge's Correction = 0.493, 95% CI= 0.006 – 0.967) (Figure 3). The data analyzed included an extra male participant ($n = 18$) compared to the rest of the analysis ($n = 17$), as one participant was unable to perform the V4 exercise protocol due to an injury but was nevertheless able to provide a sample post supplementation.

Submaximal testing

Oxygen uptake

No significant difference in $\dot{V}O_2$ between placebo (1718.9 ± 431.5 ml/min) and spirulina supplementation (1763.6 ± 358.6 ml/min) was reported ($p = 0.137$). The ANOVA revealed a significant effect between $\dot{V}O_2$ and time ($p < 0.001$). Post-hoc tests indicated that following spirulina supplementation, $\dot{V}O_2$ increased for the first 15-min at each 5-min time point ($p < 0.05$) before plateauing, whereas $\dot{V}O_2$ in the placebo intervention increased significantly for the first 10-min and the last 5-min ($p < 0.05$) (Figure 4). There was no significant interaction effect between supplement and time ($p = 0.185$).

Heart rate

There was no significant difference in HR between placebo (125 ± 11 bpm) and spirulina supplementation (125 ± 11 bpm) ($p = 0.936$). The ANOVA revealed a significant

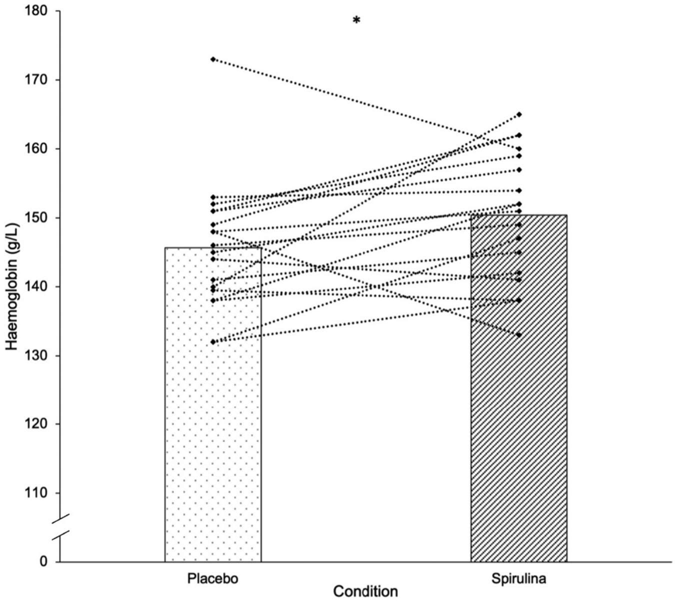

Figure 3. Hemoglobin (g/L) following placebo and spirulina supplementation. * signifies $p < 0.05$ between spirulina and placebo.

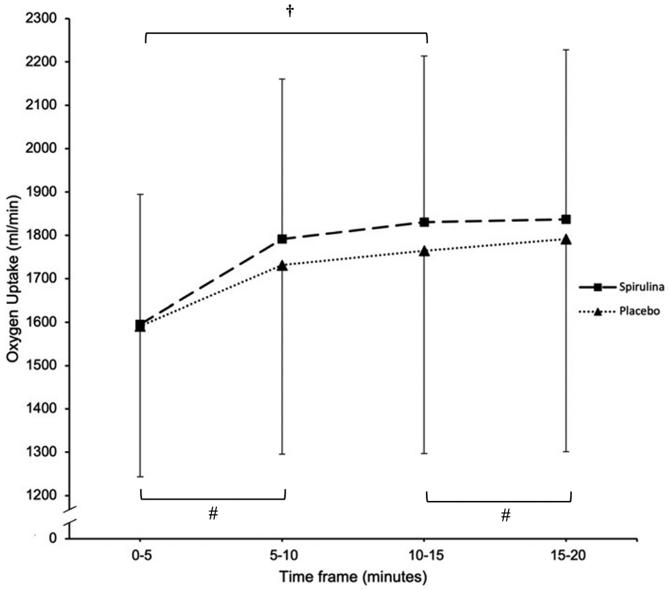

Figure 4. Oxygen uptake (ml/min) during the 20-min submaximal exercise bout following 14-days supplementation of spirulina or placebo. # signifies a significant within-trial increase in VO2 across every 5-min interval during the placebo condition $p < 0.05$ and † signifies a significant within-trial increase in VO2 across every 5-min interval during the spirulina condition $p < 0.05$.

effect between HR and time ($p < 0.001$). Post-hoc testing indicated that following spirulina supplementation, HR increased at each 5-min time point ($p < 0.05$), whereas following placebo, HR also increased at each 5-min timepoint but reached a plateau in the last 5-min ($p = 0.067$) (Figure 5). There was no significant interaction effect between supplement and time ($p = 0.623$).

RER

No significant difference in RER between placebo (0.89 ± 0.04) and spirulina supplementation (0.91 ± 0.05) was reported ($p = 0.126$). The ANOVA revealed a significant effect between RER and time ($p < 0.001$). Post-hoc tests indicated that following spirulina supplementation, RER increased for the first 10-min at each 5-min time point following both placebo and spirulina supplementation before plateauing. There was no significant interaction effect between supplement and time ($p = 0.623$) (Figure 6).

Lactate

There was no significant difference in blood lactate response during the 20-min submaximal bout between spirulina (2.45 ± 1.11 mmol/L) and placebo supplementation (2.29 ± 1.00 mmol/L) ($p = 0.898$) (Figure 7). However, ANOVA testing revealed a significant effect for time ($p < 0.001$). Post-hoc testing displayed that following both spirulina and placebo supplementation, blood lactate levels significantly increased for the first 10-min ($p = 0.001$ and $p = 0.05$, respectively), but there was no significant difference in blood lactate response for the remainder of the submaximal exercise or during the rest period

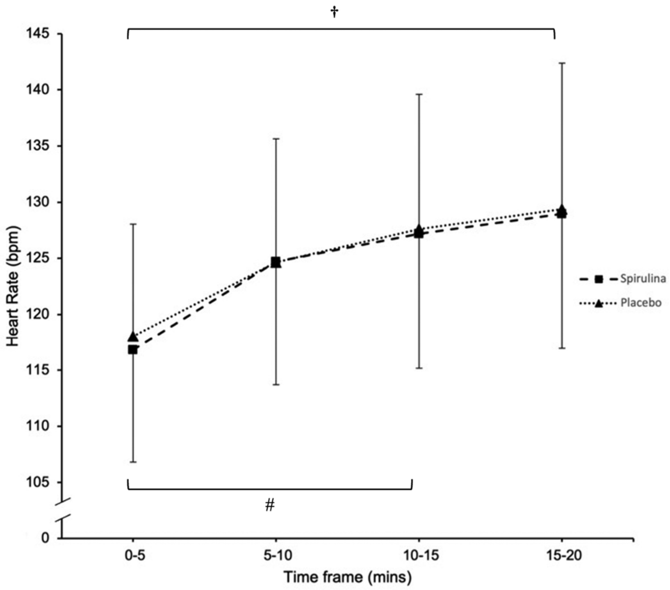

Figure 5. Heart rate (bpm) during the 20-min submaximal exercise bout following 14-days supplementation of spirulina or placebo. # signifies a significant within-trial increase in HR across every 5-min interval during the placebo condition $p < 0.05$ and † signifies a significant within-trial increase in HR across every 5-min interval during the spirulina condition $p < 0.05$.

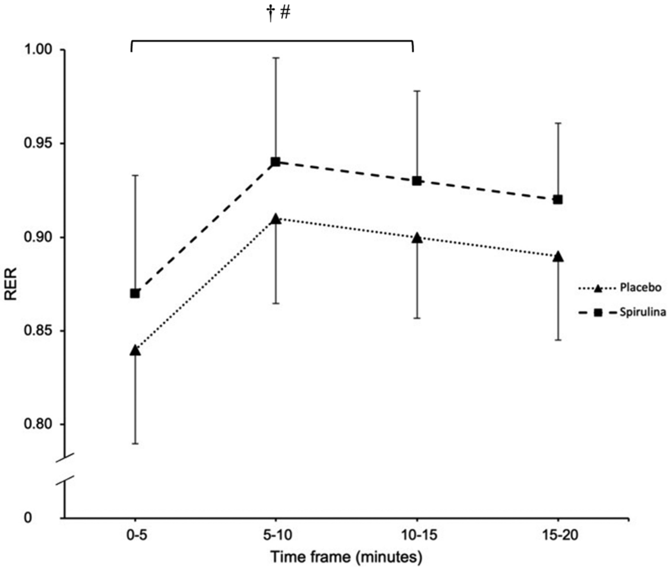

Figure 6. RER during the 20-min submaximal exercise bout following 14-days supplementation of spirulina or placebo. # signifies a significant within-trial increase in RER across every 5-min interval during the placebo condition $p < 0.05$ and † signifies a signifies a significant within-trial increase in RER across every 5-min interval during the spirulina condition $p < 0.05$.

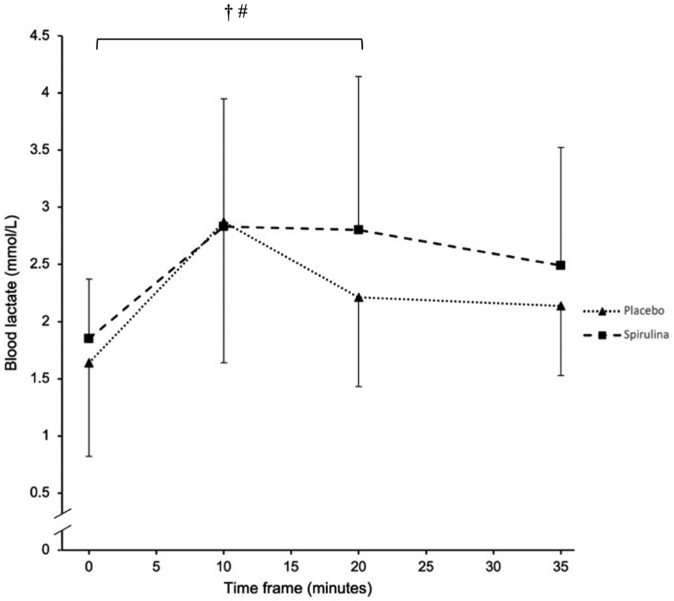

Figure 7. Blood lactate response and clearing during the 20-min bout of submaximal exercise and 15-min rest period following 14-days supplementation of spirulina or placebo. # signifies a significant within-trial increase in blood lactate across every 10-min interval during the placebo condition $p < 0.05$ and † signifies a significant within-trial increase in blood lactate across every 10-min interval during the spirulina condition, $p < 0.05$.

Table 2. Values recorded from maximal testing following supplementation periods.

	Placebo	Spirulina	p value
VO_{2max} (ml/min·kg)	44.58 ± 8.46	45.35 ± 9.58	0.454
WR_{max} (watts)	284.2 ± 61.2	291.7 ± 60.9	0.152
TtF (seconds)	503.2 ± 143.3	524.2 ± 147.0	0.105
Peak HR (bpm)	179 ± 12	179 ± 16	0.873
Peak RER	1.15 ± 0.11	1.19 ± 0.06	0.164

between the end of the 20-min bout and the start of the maximal test ($p > 0.05$). There was no significant interaction effect between supplement and time ($p = 0.053$).

Maximal testing

No variables reported any significant difference during maximal testing between spirulina and placebo supplementation (Table 2).

Discussion

This study is the first to focus on the ergogenic effects of spirulina supplementation across a 14-day period using a 6 g/day dosage. The main findings correspond with previous literature, showing increases in Hb following spirulina supplementation. Despite this, cardiorespiratory improvements in both the submaximal and maximal exercise was not observed, contrary to previous research found in 1-, 3- and 4-week supplementation periods of the same dosage (Gurney et al. 2021; Kalafati et al. 2010; Gurney and Spendiff 2020). However, the latter two of these studies involved trained participants. Although minimal, these findings prompt speculation into the mechanistic avenues of spirulina's ergogenic function and highlight the limitations within both this study and existing literature.

Hemoglobin

Spirulina has been shown to exert a positive influence on blood morphological parameters, especially Hb (Milasius et al. 2009). The 4.8 g/L (3.4%) increase in Hb found within this recreationally active cohort following spirulina supplementation aligns with research conducted in both trained male (Kelkar et al. 2008; Milasius et al. 2009; Gurney and Spendiff 2020, 2022) and anemic populations (Selmi et al. 2011). The attraction in Hb augmentation in athletes arises from its pertinence in exercise; Hb's high affinity and oxygen-binding capacity are pivotal in oxygen transport (Otto et al. 2013; Benner et al. 2021) which is integral to exercise performance (Wagner 2000; Otto et al. 2013; Hinton 2014). However, whether a 3.4% increase in Hb concentration is meaningful from an exercise performance perspective may depend on the individual's training status which is discussed further below (Schmidt and Prommer 2008). Literature has shown increases in Hb of 9.4 g/L and 10.4 g/L at the same 6 g/day dosage across a 21-day and 7-day supplementation protocol in trained and untrained individuals, respectively (Gurney et al. 2021; Gurney and Spendiff 2020). A non-significant increase of 0.84 g/L was found following a 14-day supplementation period of 2.25 g/day spirulina in high performance sportsmen (Milasius et al. 2009) and across the same timeframe, a significant 1.65 g/L increase was found following 4 g/day supplementation (Kelkar et al. 2008). Furthermore, reports showed that greater Hb improvements were found in those with lower initial Hb values (Kelkar et al. 2008). Similar to this intervention, where the top 50% of participants who had the greatest increases in Hb had an initial measurement that was on average 4 g/L lower than the bottom 50% who experienced smaller increases in Hb. Thus, suggesting that spirulina supplementation may be more beneficial in those with poorer nutritional status. Comparison of results between studies is difficult given the differing populations, training and nutritional statuses, however the notion that spirulina supplementation increases Hb is seemingly well supported. Thus far, spirulina's nutritional composition is suggested to be explanatory for the increase in Hb. Literature widely alludes to the presence of iron in spirulina (Milasius et al. 2009), which is reported to have high bioavailability, fundamental in increasing Hb synthesis (Selmi et al. 2011; Kalpana et al. 2017; Gurney and Spendiff 2022).

VO_{2max} testing

This study found no improvements in $\dot{V}O_{2max}$ cycling following a 14-day supplementation period. However, longer supplementation protocols investigating lower-limb exercise modalities reported improvements in time to exhaustion across a 4-week period at 6 g/day (Kalafati et al. 2010) and $\dot{V}O_{2max}$ over a 6-week period but using a smaller 4.5 g/day dosage (Hernández-Lepe et al. 2018). Thereby indicating that a longer supplementation period may be optimal to derive a positive ergogenic response in lower-limb exercise. Only one study to date, following a week-long supplementation protocol, found improvements in $\dot{V}O_{2max}$ during upper-body arm-cycling (Gurney and Spendiff 2020). The contrasting results between supplementation timeframes and exercise modalities could be explained by the different physiology surrounding hemodynamics and muscle fiber types between upper- and lower-limb musculature as well as the plausible blood morphological adaptations onset by spirulina supplementation.

The correlation between Hb and $\dot{V}O_{2max}$ is well reported (Prommer et al. 2018). As such, for every 1 g/L increase of Hb, $\dot{V}O_{2max}$ can purportedly increase by approximately 3 ml/min (Schmidt and Prommer 2008) – which may reflect an improvement in cardiovascular exercise performance due to the imperative role of Hb in oxygen transport. Yet despite a 4.8 g/L increase in Hb, a corresponding increase in $\dot{V}O_{2max}$ was not reported in this study. A variety of other physiological factors, particularly mitochondrial oxygen uptake, could present as the limiting factor to the potential ergogenic aid secondary to increasing Hb concentration (Wagner 2000). Moreso, studies have shown that acute changes in oxygen provision do not significantly impact exercise capacity in untrained individuals (Wagner 2000), thus implying that an increase in Hb may not significantly benefit $\dot{V}O_{2max}$ amongst recreationally active individuals. This further alludes to metabolic factors, such as mitochondrial oxygen uptake, being the predominant cause of limitation in this cohort's $\dot{V}O_{2max}$ (Bassett and Howley 2000; Wagner 2000).

In addition, the difference in results between the above studies could also be due to the variable training and nutritional status of participants, as the more trained and nutritionally adequate the participant, the slower the progress curve and rate of improvement, and in some cases no improvement (Franca et al. 2010). For example, the participants in the arm-cycling study (Gurney and Spendiff 2020) were untrained and unfamiliar with the exercise modality, similar to participants in another lower-limb based exercise protocol, who were sedentary and overweight (Hernández-Lepe et al. 2018), yet significant improvements were observed. The rate of $\dot{V}O_{2max}$ improvements in untrained or sedentary groups by supplementing with spirulina may therefore differ to those already experienced and accustomed to running/cycling.

Submaximal testing

No changes in HR, $\dot{V}O_2$, RER and lactate were reported following spirulina supplementation. Intensities below 50% $\dot{V}O_{2max}$ appear inadequate in evoking improvements in cardiorespiratory fitness amongst healthy adults (Swain and Franklin 2002), and according to the American College of Sports Medicine guidelines, 40% is considered an intensity insufficient to surpass the minimum threshold of intensity to elicit any ergogenic effect (Garber et al. 2011). This study demonstrated a significant increase in HR within both the placebo and spirulina interventions for the first 15-min. This can be attributed to the increase in core body temperature and sympathetic activity (Souissi et al. 2021). The findings of this study conflict with existing literature as two studies investigating a 6 g/day dosage, at an intensity of 55% $\dot{V}O_{2max}$ in submaximal exercise found a significant reduction in HR both in a 30-min arm-cycling protocol (149 ± 18 bpm spirulina vs 154 ± 14 bpm placebo) (Gurney and Spendiff 2020) and a 60-min bout of cycling (139 ± 11 bpm spirulina vs 144 ± 12 bpm placebo) (Gurney et al. 2021), following 1-week and 3-week supplementation periods, respectively, suggesting reductions in homeostatic disturbances during submaximal intensities post-supplementation. This was also confirmed when comparing HR recovery values following a cycling $\dot{V}O_{2max}$ test (Kalpana et al. 2017). Components such as arginine and phycocyanin, both present in spirulina, have been speculated as physiological mechanisms of action for this reduction in HR due to their vasodilatory function. Arginine, an essential amino acid, increases nitric oxide bioavailability (Lafarga et al. 2020) which consequently improves endothelium-dependant vasodilation *via* the intracellular second-messenger cGMP (Bode-Böger et al. 1996). Whilst phycocyanin has been found to increase the expression of endothelial nitric oxide synthase in rats (Ichimura et al. 2013). Although further human trials are needed, the increase in nitric oxide bioavailability could suggest an enhanced oxygen provision to skeletal muscle resulting from the improvements in blood flow (Gurney and Spendiff 2020) achieved *via* increases in vascular reactivity and decreases in contractile reactivity. It could therefore be speculated that spirulina might function as a protective mechanism by minimizing the heart's workload during submaximal exercise whilst providing the skeletal muscles engaged in exercise with adequate oxygen. Albeit further research needs to be undergone to establish said mechanisms, especially as the findings are not consistent.

No changes in the blood lactate response were observed in this study following spirulina supplementation, which conflicted with existing literature found across a variety of populations, notably in trained cyclists, where reductions were reported (2.05 ± 0.80 mmol/L spirulina vs 2.39 ± 0.89 mmol/L placebo) at a 6 g/day dosage with 21-days supplementation (Gurney et al. 2021). Moreover, a 6-week study investigating 4.5 g/day in sedentary, overweight, and obese adults displayed a prolonged onset of blood lactate accumulation post-supplementation (Hernández-Lepe et al. 2018), whilst an insignificant decrease was found in recreational runners pre- (1.7 ± 0.6 mmol/L vs 2.3 ± 2.2 mmol/L) and post-exercise (12.8 ± 6.3 mmol/L vs 15.5 ± 4.2 mmol/L), following a 5 g/day 15-day protocol (Torres-Durán et al. 2012). Furthermore, Lu et al. found a decrease in pre-exercise resting lactate in untrained college students (20.40 ± 3.01 mmol/L vs 27.29 ± 3.16 mmol/L) (Lu et al. 2006).

The submaximal results from the current study imply that 20-min and/or a 40% intensity are inadequate in evoking sufficient metabolic stress to prompt the requirement of an ergogenic aid, or perhaps the increase in hemoglobin was not sufficient to improve oxidative metabolic capacity to allow for a reductions in HR and blood lactate response as hypothesized by Gurney et al. (2021). Longer and more intense exercise protocols are more prone to inducing fatigue *via* the increased accumulation of metabolites such as lactate, hydrogen ions, and inorganic phosphate in skeletal muscle cells, resulting in metabolic acidosis and a reduction in intracellular oxygen levels (Freitas et al. 2017). The purported antioxidant and blood morphological changes onset by spirulina supplementation may also provide a protective effect and be of more use in protocols of greater intensity. Therefore, a longer or more intense bout of steady-state exercise may be required to uncover any potential effects of spirulina.

The RER results from the current study further support previous studies by Gurney et al. where there were also no changes in RER during a 30- and 60-min bout of submaximal exercise (Gurney et al. 2021; Gurney and Spendiff 2020). However, these results conflict with Kalafati et al. who reported a decrease of 10.3% in carbohydrate oxidation and 10.9% increase in fat oxidation during a 2-h run at 70–75% $\dot{V}O_{2max}$ in trained runners (Kalafati et al. 2010). Due to spirulina's rich composition, it is difficult to identify the exact underlying biochemical mechanism, however the high content of F-linolenic acid in spirulina have been suggested to play a role in mediating fat metabolism (Kalafati et al. 2010). A potential physiological explanation in such conflicting findings could lie in the intensity and duration of the protocols; with lipid oxidation being preferential in longer durations of exercise (>1 h), and intensities of 45–65% $\dot{V}O_{2max}$ (Purdom et al. 2018). Given the short, low intensity nature this submaximal cycle, it is possible that the intensity/duration of exercise was insufficient to cause lipid oxidation to increase. Thereby consolidating the notion that spirulina may be preferential for more intense or longer periods of submaximal activity.

Existing research displays a mixture of results following spirulina supplementation, potentially arising from the variations in training status and exercise protocols used. Despite reports of positive findings, the amount and type of exercise undergone during supplementation periods was not controlled in several studies and the performance outcomes were only measured by a single bout of exercise (Lu et al. 2006; Kalafati et al. 2010; Kalpana et al. 2017; Gurney and Spendiff 2020). Variable participant's training regimes present as confounding factors and could affect the outcome measures following supplementation. Gurney et al. requested maintenance of regular training regimes and still found improvements across some cardiovascular parameters (Gurney et al. 2021). However, Franca et al. investigated cyclists with a higher training volume and load and controlled the intensity of every training session (Franca et al. 2010). Despite this, no exercise performance test was conducted, only oxidative stress biomarkers were measured, and despite using a higher dosage of spirulina (7.5 g/day), no ergogenic improvements regarding muscle proteolysis and oxidative stress were detected across a 28-day supplementation period (Franca et al. 2010). Yet, during a supplementation protocol where 7.5 g/day was ingested for 21-days in untrained students, improvements were recorded in those exact biomarkers (Lu et al. 2006). The research surrounding the physiological adaptations following spirulina supplementation, especially upon comparing trained and untrained individuals, is scarce. However, it has been claimed that dietary status can affect the extent of adaptation following spirulina supplementation (Franca et al. 2010; Gurney and Spendiff 2022).

Lastly, as spirulina is a multicomponent species, it is worth noting its potential capability regarding antioxidant activity which may contribute to providing ergogenic aid by relieving oxidative stress (Wu et al. 2016). Despite the equivocal evidence regarding spirulina's influence on human redox status, its composition should still be taken into consideration, especially its cysteine levels (0.45 g per 100 g) which are essential for optimal redox status and muscle function (Gurney and Spendiff 2022).

Limitations

A potential limitation of the study is that the incremental exercise protocol was preceded by submaximal exercise. Although it was at a very low intensity, the submaximal exercise bout may have induced fatigue and consequently affected $\dot{V}O_{2max}$ and maximal power output, particularly due to the varied trained status of participants.

Furthermore, the benefits derived from spirulina's nutritional composition need to be distinguished in individuals with varying degrees of nutritional status, as this may have been the factor distinguishing 'responders' from 'non-responders' (Gurney et al. 2021). A more thorough nutritional anamnesis and blood test assessing clinical and biochemical parameters could address this issue as it would remove the need and inaccuracy of using a 3-day dietary log; however, this was beyond the resources available for this study.

Moreover, despite instructing participants to maintain their regular training regimes, and requesting the submission of 3-day activity and nutritional logs prior to each visit, stronger control and monitoring of physical activity and diet throughout the intervention could have been instilled. This was demonstrated well by Franca et al. (2010), where stricter monitoring of training and diet was employed across the entire protocol. Daily monitoring to this degree was not feasible in this study, due to the generally unstructured and varied nature of a recreationally active individual's training (McKay et al. 2022).

Another potential limitation, and one present in all of the aforementioned studies regarding spirulina supplementation in a randomized controlled trial format using the double-blind protocol, is that the turnover rate of hemoglobin being greater than 100 days, could deem the washout periods to be ineffective, thus skewing the results especially for those allocated spirulina first in the intervention – however, Kashani et al. reported that there was no carryover effect following a 14-day washout period (Kashani et al. 2022).

Underrepresentation of female athletes is also still an issue within nutritional intervention studies. Only three females were included in this study; a greater female population needs to be targeted to increase the external validity of this research (Gurney et al. 2021). Exploring gender differences is an avenue of paramount importance in exercise performance and supplementation due to the physiological differences, especially surrounding cardiac output, Hb levels, and oxygen uptake (Lundsgaard et al. 2017).

Conclusion and future directions

To conclude, 14-days of spirulina supplementation elicits a small but significant improvement in Hb yet does not result in any substantial ergogenic benefits amongst recreationally active individuals during submaximal and maximal cycling. Nonetheless, this study continues to tackle the paucities in literature regarding spirulina supplementation as a prospective ergogenic aid, addressing gaps surrounding spirulina's optimal usage. Despite this, spirulina should continue to be investigated across different dosages, time periods and intensities of exercise, to help identify a potential optimal dose/duration for submaximal and maximal exercise as well as further understand its underlying mechanisms of activity. Future research should aim to include the female population to a greater extent and prioritize novel dose/durations alongside the use of nutritional and training matching throughout the course of the intervention.

Authors' contributions

The study was designed by Y. Ali, R. Aubeeluck and T. Gurney; data were collected by Y. Ali and R. Aubeeluck; data analysis and interpretation was undertaken by Y. Ali and R. Aubeeluck; manuscript preparation was undertaken by Y. Ali, R. Aubeeluck and T. Gurney. All authors approved the final version of the paper.

Disclosure statement

No potential conflict of interest was reported by the authors.

Ethical approval

The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation in any way to intentionally portray anything but those outcomes that were observed. The Ethics Committee at University College London approved the study (21745/001) in accordance to the Helsinki Declaration. All participants completed informed consent before participating in the study.

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Data availability statement

Data generated or analyzed during this study are available from the corresponding author upon request.

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