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ORIGINAL ARTICLE

Photobiomodulation by light emitting diode applied sequentially does not alter performance in cycling athletes

Gabriela de Carvalho[1] · Aline Gobbi[1] · Ronaldo Bucken Gobbi[1] · Damião Miranda Ngonga Alfredo[1,2] · Thales Henrique do Carmo Furquim[1] · Rafael Inácio Barbosa[1] · Marcelo Papoti[1] · Rinaldo Roberto de Jesus Guirro[1,3]

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Abstract

Analyze the effects of sequential application of photobiomodulation therapy (PBMT) at different wavelengths on the performance of cycling athletes. Cyclists (48 male, mean age 33.77 years) underwent a performance evaluation through an incremental test, VO2max, blood lactate analysis, perception of effort, infrared thermography, and isokinetic evaluations. Photobiomodulation (180 J) with infrared (IR 940 ± 10 nm), red (RED 620 ± 10 nm), mixed Red, and IR (RED/IR 620 + 940 nm) or Sham (disabled device) intervention occurred on three consecutive days and was applied to the quadriceps femoris bilaterally. Reevaluations were performed 24 h after the last application, with 1 week of follow-up. A significance level of 5% was adopted, and the effect size (ES) was calculated by Cohen's d. Results: There were no significant differences in the analyzed variables under any experimental condition ($p > 0.005$), but a moderate effect size was observed for torque peak at 60°/s on left lower limb (LLL) (ES = 0.67), average power at 60°/s of the right lower limb (RLL) (0.73), and LLL (ES = 0.65) and a considerable effect size in torque peak at 60°/s of the RLL (ES = 0.98) in the IR/RED group compared with sham 24 h after the last application. Moreover, a large effect size was observed for total time to exhaustion (ES = 1.98) and for VO2max (ES = 6.96), and a moderate effect size was seen for anaerobic threshold (ES = 0.62) in the IR/RED group compared with sham. Photobiomodulation, when not associated with training, was not able to produce a cumulative effect on the performance of cycling athletes. However, the association of two wavelengths seems to be better for increased performance. ClinicalTrials.gov Identifier: NCT03225976

Keywords Performance · Athletes · Fatigue · Photobiomodulation · Clinical trial

Introduction

It is known that photobiomodulation therapy (PBMT) is capable of generating intracellular modifications by increasing mitochondrial metabolism [1]. This increase is related to the absorption of light by chromophores, which are mostly concentrated in cytochrome C oxidase (Cox), present in complex IV of the electron transport chain [2]. Such absorption generates the photodissociation of the nitric oxide of this complex, causing an increase in activity and consequently higher synthesis of ATP [3].

Based these mechanisms, benefits can be seen in a range of outcomes discussed in recent systematic reviews, in which promising results in terms of increasing muscle strength [4], increased time to exhaustion, and decreased fatigue are prevalent [5, 6]. It is also possible to discuss the parameters of photobiomodulation to provide better results, as Ferraresi et al. [4] presented a therapeutic window of 56 to 315 J for the femoral quadriceps. Similarly, Vanin et al. [7] observed very high values in their review, with a therapeutic window of the ideal dose of 60 to 300 J for large muscle groups.

Clinical trials have shown that LED photobiomodulation is a beneficial technique regarding muscular strength capacity, in relation to an increase in maximum voluntary contraction and peak torque [8–12]. As described in a study by Vanin et al. [13], where red and infrared LEDs were applied before an exercise protocol was performed in healthy individuals,

✉ Rinaldo Roberto de Jesus Guirro
rguirro@fmrp.usp.br

[1] Department of Healthy Science, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil

[2] Angola Methodist University, Luanda, Angola

[3] Ribeirão Preto Medical School, University of São Paulo, Avenida Bandeirantes, 3900, Ribeirão Preto, São Paulo 14049-900, Brazil

showing that photobiomodulation was able to increase the maximum voluntary contraction of these individuals. In addition, the delay to the beginning of fatigue and the reduction in cellular damage due to photobiomodulation is due to the greater energy supply, reduction in oxidative stress [14, 15], decreased levels of lactate (CK), and C-reactive protein, demonstrating a protective effect against muscle damage and fatigue [11, 12, 16–18].

Due to its physiological, therapeutic, and biochemical effects, it is possible to observe in some studies [9, 19] that LED promotes an increase in the performance of healthy individuals, with an improvement in the distance performed, increase in time to exhaustion and gain in VO2max. However, although its effects are well-established in the literature, there is a complex diversity of dosages, wavelengths, therapeutic windows, and application models [20].

Clinical trials using LEDs as a source of light describe in their methodology the association of two wavelengths, i.e., red and infrared (IR), in the same application to achieve the desired ergogenic and protective effects. However, there is no justification in the literature to support the claim that the combined use of these two wavelengths is the best method of applying LED photobiomodulation [21].

The time between application and the subsequent mitochondrial effects is also much discussed in the literature [22]. Passarella et al. [23] suggest that the time between the application of the therapy and the increase of mitochondrial metabolism occurs in 3 min. Karhu et al. [24], in their in vitro study, observed an increase in ATP synthesis within 25 min after the application of photobiomodulation. More recent studies [25, 26] observed modifications of Cox in a time window of 5 to 24 min after muscle irradiation. Conversely, De Brito et al. [27] and Ferraresi et al. [28] state that the application of photobiomodulation therapy generates a tissue effect in 24 to 48 h.

These results appear to be conflicting, so Ferraresi et al. [4] raised a question about a possible cumulative effect of this technique when applied repetitively. So far, the literature does not present any study that investigated this type of application, with standardization of the parameters testing an increase in performance and an investigation of the best wavelength to be used. Therefore, the objective of this study was to analyze the acute and chronic effects of the sequenced application of LED photobiomodulation at different wavelengths on the fatigue and strength abilities of cycling athletes.

Methods

Study design

This was a blinded, placebo-controlled randomized clinical trial. The athletes were evaluated in two sessions at the same time on separate days and submitted to three consecutive days of LED photobiomodulation to the quadriceps femoris muscle, bilaterally, with a 24-h interval between each application. The evaluations of performance aspects for all participants were performed in a controlled environment in the following order: warm-up, isokinetic dynamometer, 30-min interval, incremental test, physiological evaluations, and infrared thermography; this was followed by 3 days of LED application and a re-evaluation. For the strength evaluation by the isokinetic dynamometer, a follow-up of 1 week was performed to observe possible residual effects (Fig. 1).

Randomization and allocation of athletes was performed by a researcher who did not participate in interventions, assessments, and data collection. Randomization was performed at the beginning of the project, divided into the following groups: Sham (disabled device), RED LED (620 ± 10 nm), IR LED (940 ± 10 nm), and RED/IR LED (620 + 940 nm) homogeneously. A numeric table was generated using Excel software to create random numbers, which were placed in opaque and sealed paper envelopes, opened only in the presence of the volunteer. Interventions were performed by researcher 1, as well as all evaluations were performed by researcher 2, blinded to the group, both trained before the beginning of data collection. Data analysis and processing was performed by researcher 3, also blindly (Fig. 2).

The study was approved by the institution's ethics committee (2,137,629), and informed consent was obtained from all individual participants included in the study. The study was also enrolled in ClinicalTrials.gov (NCT03225976).

Population

The sample size was based on a study by Antonialli et al. [10], using the quadriceps strength difference between the groups, considering 61.34 N/m and a standard deviation of 26.87 N/m, which resulted in a minimum of 10 athletes per group (power of 95%, alpha 0.05). Considering 20% loss, 48 male amateur cycling athletes participated in the study. All athletes were healthy individuals, regular practitioners of regional and national cycling events, without musculoskeletal injury or cardiorespiratory disease in the last 6 months, and did not use anabolic steroids. The participants were recruited in the city of Ribeirão Preto, SP, Brazil, and region, through social media. Participants who had any of the injuries as mentioned above, illnesses, or those who used any illicit substance or anti-inflammatory or analgesic drugs during the data collection period were excluded from the study.

Because it is an individual sport, and a study that sought to investigate the effects of photobiomodulation not associated with training, individuals did not perform their usual training during the first week of collection to eliminate any bias regarding the individual training volume of each athlete.

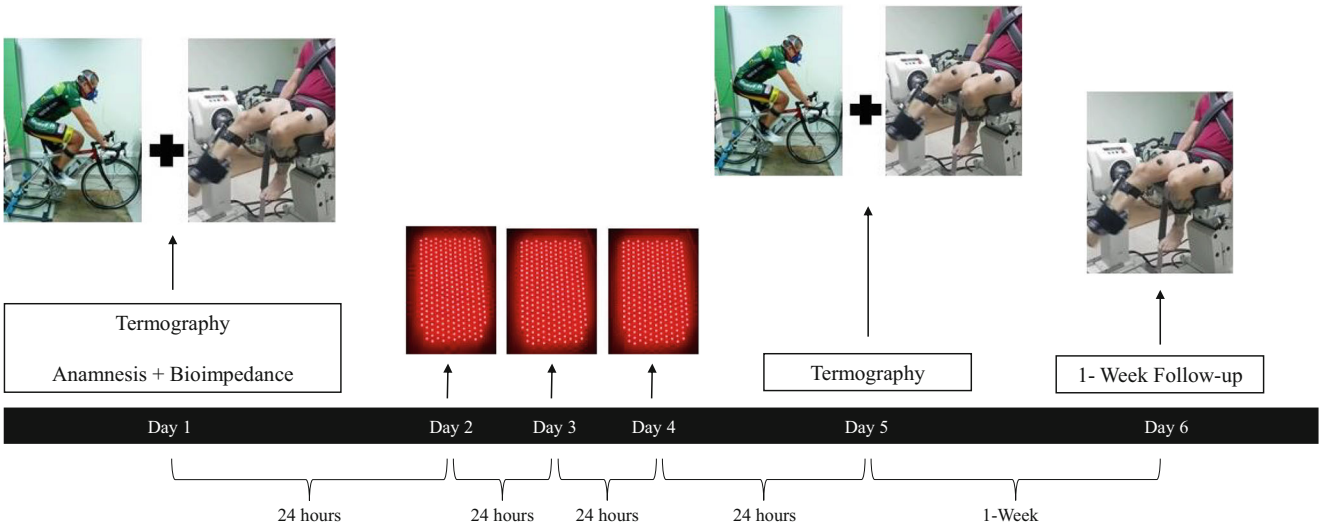

Fig. 1 Chronological representation of evaluations and interventions

Isokinetic dynamometer

Before the test, a 5-min. exercise cycle was performed on a bicycle with a 60-W load and a constant speed of 15 km/h. The athletes were properly positioned and stabilized by belts securing the trunk, pelvis, and distal third of the thigh in a Biodex System 4 Pro isokinetic dynamometer (Biodex Medical Systems®, New York, USA) (ICC = 0.98) [29]. A familiarization of 10 concentric isokinetic contractions at 60°/s and 270°/s was performed. The athlete then performed five concentric maximal isokinetic contractions at an angular velocity of 60°/s. After 90 s of rest, the athlete performed 30 more concentric isokinetic contractions at the angular velocity of 270°/s, both with a total range of motion of 90°. The test was done with both lower limbs. The participant was verbally encouraged by a single, trained evaluator throughout the test. Peak torque, mean power, and the agonist/antagonist ratio (I:Q) were evaluated. The re-evaluation occurred 24 h after the last LED application, with follow-up after 1 week.

Incremental test

The cyclists were submitted to an incremental test until volitional exhaustion, for the determination of metabolic thresholds, maximum oxygen consumption (VO2max) (ICC = 0.88) [30], and intensity corresponding to VO2max (iVO2max). The test was performed on a stationary roller (Tacx ™ Fortius Multiplayer, Wassenaar, Netherlands) that allows for maintenance of the predetermined power (W) for each stage, regardless of the cadence used by the athlete. The stationary roller was attached to the bicycle of each athlete, making the evaluation closer to training. The initial power was 100 W with increments of 30 W every 2 min. The increment was applied to exhaustion [31, 32]. Each volunteer was timed to determine iVO2MAX if the participant did not complete the internship.

Gas analysis

The ventilatory and oxygen consumption variables were constantly monitored every three breaths using a gas analyzer (VO2000 Inbramed, Rio Grande do Sul, Brazil). The variables of interest corresponding to each stage were expressed using the last 30 s. VO2max was assumed to be the highest value observed at the end of the incremental test (mean of the last 30 s). The subjective perception of effort (PSE) was determined at each stage by the Borg effort perception scale of 10 points.

Blood analysis

Before the incremental test, at the end of each stage, 25 μL of ear lobe arterial blood was collected in heparinized capillaries and stored in Eppendorf-type polyethylene tubes (1.5 mL) containing 50 μL of sodium fluoride (NaF) to determine the concentration of blood lactate [33], analyzed using a lactometer (Yellow Springs Instruments 2300, Ohio, USA). Lactate values are expressed in mmol/L. The intensities corresponding to the lactate threshold (iLL) and the anaerobic threshold (iLAN) were determined by the two inflections of the bi-exponential adjustment of the power ratio by the blood lactate concentration.

Thermography

For the thermographic evaluation the instrument was stabilized for 10 min before the test in a controlled environment (22° ± 2 °C and humidity of 50%) emissivity of 0.98 [34], and the athletes were instructed to avoid, in the previous 2 h, hot

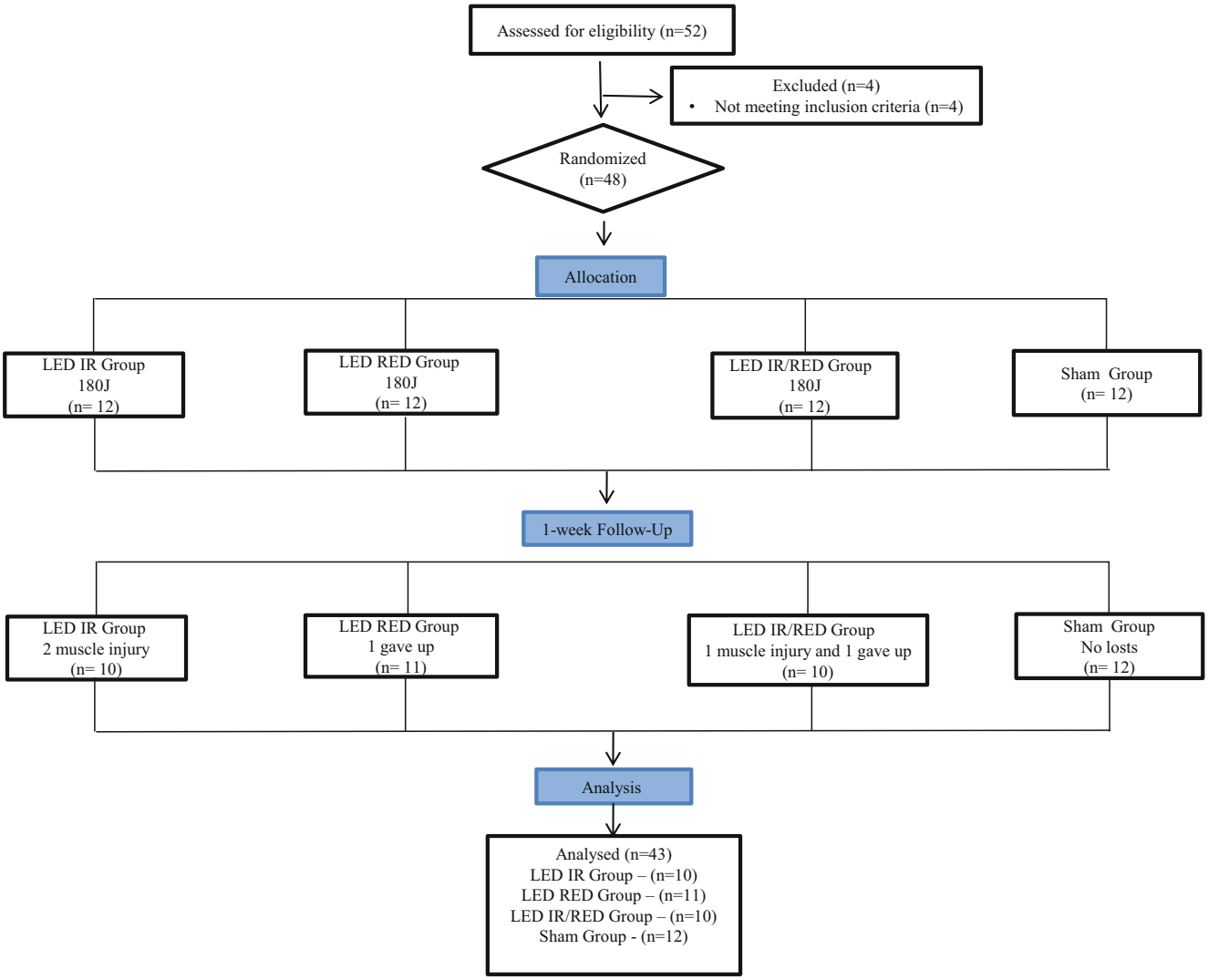

Fig. 2 Study flowchart

baths, vigorous exercise, use of creams or ointments, and the ingestion of stimulant foods such as caffeine, nicotine, and chocolate. To capture the incremental pre-test image, the athletes remained seated for 15 min in an environment with the controlled temperature at 22° ± 2 °C [35]. During the capture of the images, the athletes remained in an orthostatic position, and the place photographed free of clothes. A model T300 thermal camera (FLIR® Systems, Danderyd, Sweden) was used with accuracy up to 0.05 °C. Three infrared images were captured in sequence, at a distance of 100 cm from the volunteer, in order to allow the framing of the muscles to be evaluated [36]. To determine the value of muscle temperature, the software QuickReport, version 1.2 (FLIR® Systems) was used, where two regions of interest (ROIs) were evaluated, i.e., the right and left thigh. The images were taken before the beginning of the evaluation and immediately after the incremental test.

Photobiomodulation

The application of photobiomodulation occurred on the second, third, and fourth day of collection, 24 h after data collection at baseline. The therapy was applied using a three different LED devices developed at the institution, with the aid of the Biophotonics Laboratory of the Medical Physics Course (FFCLRP-USP), with dimensions of 25 × 42 cm^2, with equidistant distribution between the LEDs (1 × 1 cm) (Fig. 2) and a total energy of 180 J. The therapy was applied bilaterally on the quadriceps femoris muscle. All the LEDs were calibrated before the beginning of the application, in which the wavelength, power, and power density were checked (Table 1).

For the application of Sham group photobiomodulation therapy, the device was connected to a deactivated power supply. However, the participants had the auditory stimulus of the on and off switch, so that they believed they were being

irradiated. A total of 0 J was irradiated in each member of the athletes allocated to this group.

Statistical analysis

Statistical analysis was computed using SPSS for Windows version 2.0 (SPSS, Inc., Chicago, IL). Descriptive statistics was used to describe the means and standard deviations of the participants' characteristics. Adjusted between group mean differences (MD) and 95% confidence intervals (CI) were calculated using linear mixed models by using group-by-time interaction terms. Moreover, Bonferroni correction test was used to compare between the groups. The *P* value was set at 0.05. The effect size was determined by Cohen's d, and the interpretation of the values was based on the classification established by Cohen (1992): less than 0.2 as a small effect, around 0.5 as a moderate effect, and above 0.8 as a large effect.

Results

A total of 48 individuals participated in the study. In the follow-up period, five participants did not perform the reevaluations, three due to musculoskeletal injuries, and two due to abandonment. The results were not impaired since the values remained within the sample loss (Fig. 4). There was no significant difference between groups at the time pre-assessments for any of the variables analyzed ($p > 0.05$). The characteristics of the study participants are presented in Table 2.

The torque peak of the quadriceps 60°/s, power 60°/s, I:Q 60°/s and 270°/s, of the MID and MIE did not present differences in post-LED moment ($p > 0.05$, Table 3). However, a significant difference ($p < 0.05$, Table 3) can be observed for the Red/IR Group compared with the Sham and the IR group at the post-LED moment, in the peak torque and average power at 270°/s in both limbs. Statistical difference ($p < 0.05$, Table 3) was also observed for the Red/IR Group compared to Red at 270°/s (MID) and mean power at 270°/s (MIE). No variable showed statistical difference at 1 week follow-up ($p > 0.05$, Table 4). Moderate effect size was observed for torque peak at 60°/s on left lower limb (MIE) (0.67), average power at 60°/s of the right lower limb (MID) (0.73) and MIE (0.65), and a large effect for torque peak MID (0.98) in the IR/RED group compared with sham 24 h after the last application. Large and moderate effects could also be observed when comparing the IR/RED group to the RED at torque peak at 60°/s MID (1.03) and MIE (0.88), torque peak at 270°/s MID 0.54) and MIE (0.64), mean power at 60°/s MID (0.63) and MIE (0.61), as well as when compared with the IR group at torque peak at 60°/s MID (0.62) and MIE (0.60), average power at 60°/s MID (0.86) and MIE (0.60), torque peak at 270°/s MID (0.62) and MIE (0.51) and in the average power at 270°/s MID (0.68) and MIE (0.63).

There were no significant differences in the performance of the athletes regarding total time to exhaustion, VO2max, iLAN, and skin temperature ($p > 0.05$) in all groups. Although no statistical differences were observed, the IR/RED group compared with the sham group demonstrated a large clinical effect for the total time to exhaustion (1.98) and for VO2max (6.96), and a moderate clinical effect for iLAN (0.62). As well as, when compared with the IR group, large effect size was observed for VO2max (1.02), moderate for total time to exhaustion (0.69), and also when compared with the RED group, where a moderate effect size for total time to exhaustion (0.74) and iLAN (0.49). The PSE of the athletes did not have a significant influence regardless of the intervention, regarding the total test time (Fig. 3).

Discussion

This was the first study to investigate the effects of sequential application of photobiomodulation therapy as muscle preconditioning when not associated with strength or endurance

Table 1 Characterization of the LEDs that make up the devices blankets

Variables	Blanket RED	Blanket IR	Blanket IR/RED
Wavelength (nm)	620 ± 10	940 ± 10	940 ± 10 + 620 ± 10
Number of LEDs	267	267	141 IR e 126 RED
LED diameter (cm^2)	0.125	0.178	0.178/0.125
Blanket diameter (cm^2)	25 × 42	25 × 42	25 × 42
Power density (mW/cm^2)	52.86	33.70	33.70/52.86
Power/diode (W)	0,006	0.006	0.006
Power output (blanket) (W)	1.76	1.60	1.67
Energy/diode (J)	0.61	0.64	0.64/0.61
Total energy (J)	360 (180/quadriceps)	360 (180/quadriceps)	360 (180/quadriceps)
Irradiation time (s)	102	108	153

Table 2 Baseline characteristics of the participants

Variables	Sham group	RED group	IR group	IR/RED group
Age (years)	34 ± 7.33	33.75 ± 8.25	34.25 ± 8.11	31.91 ± 7.08
Mass (Kg)	81.2 ± 10.04	77.01 ± 6.97	78.41 ± 9.57	77.99 ± 9.19
Stature (cm)	1.77 ± 0.08	1.73 ± 0.08	1.74 ± 0.04	1.77 ± 0.04
IMC (Kg/m^2)	25.70 ± 2.08	30.02 ± 6.34	30.43 ± 6.34	24.55 ± 2.37
Fat free mass (Kg)	63.90 ± 8.02	61.05 ± 7.13	62.69 ± 7.54	64.44 ± 6.03
Skeletal muscle mass (Kg)	36.29 ± 4.71	34.48 ± 4.26	35.49 ± 4.58	36.5 ± 3.50
Fat percentage (%)	20.70 ± 6.18	20.55 ± 6.04	19.56 ± 5.93	16.70 ± 4.82
Years of experience in cycling	5.75 ± 4.95	6.75 ± 8.77	7 ± 6.80	7.20 ± 6.16
Trainings per week	3.91 ± 0.99	4 ± 0.95	4.16 ± 1.33	4.83 ± 1.19

training at different wavelengths. Our initial hypothesis assumed that the sequential application of photobiomodulation could generate cumulative effects and optimize the application of this therapy for performance enhancement in cycling athletes. However, our main findings do not support our initial hypothesis, suggesting that it is not possible to observe cumulative effects of PBMT when not applied in combination with training or immediately before physical exertion.

Studies show that the application of PBMT can increase the maximum voluntary contraction of healthy individuals when applied before training, or as a recuperative technique [12, 13], as well as in reduction in time to exhaustion, the reduction in oxidative stress, and the decrease in biomarkers of muscle damage [6, 11, 12]. Our results contradict most studies found in the current literature. However, there is an important methodological difference in the application of PBMT, which may have a great influence on the results obtained in our study.

Photobiomodulation can be considered an ergogenic resource and is very well consolidated in the literature. Its effects are directly related to increased ATP synthesis by the absorption of the photons released by PBMT and their absorption by the chromophores present in cytochrome c-oxidase, inside the electron transport chain; consequently there are decreased oxidative stress increased local microcirculation, [5, 28]. Several systematic reviews recommend its use, despite the issues involved in the ideal parameters [4, 7]. Leal-Junior et al. [37] published a masterclass of recommendations for standardization in the use of the parameters of photobiomodulation. However, some issues are yet to be elucidated, because the divergence between positive and non-positive results may be directly related to these parameters.

In addition to the divergence of parameters found in the various studies, we can also observe the application of PBMT majority in healthy individuals. It is hypothesized that there may be a difference in relation to the results obtained in this population and in individual athletes, as already observed [38]. Studies by Lanferdini et al. [20] are the most recent for the application of photobiomodulation therapy in cycling athletes. Different doses of the treatment were evaluated for assessments of the physical performance of these athletes, using evaluations very similar to those of our study. However, the authors observed significant positive changes in time to exhaustion, especially at a dose of 135 J, which is also very close to the dosage used in our study (180 J). In a second study by Lanferdini et al. [39], where VO2 kinetics were evaluated in this same population, it was not possible to observe a change in the amplitude of VO2. Despite the similarity of the population and the variables, in our study, it was not possible to observe the same gains in the performance of these athletes, since the application models differed, showing that consecutive application with a cumulative effect not associated with training seems not to be the best scenario for photobiomodulation therapy.

In addition to the above, the time between the application and the exercise to be assessed is pertinent. Peserico et al. [40] suggested in their study that short time (3–5 min) between application and exercise performance seems to not be the ideal scenario. Previous studies have raised this question and, although it is still not clarified in the literature, Ferraresi et al. [4], suggested a more extended time between the application of photobiomodulation and the exercise to be performed. The response time used in this study was relatively large compared with other studies, with a 24-h window between applications, and also between the last application and the performance evaluation of athletes (20, 38, 39, 40). According to the results obtained in this study, 24 h seems not to be the ideal time, surpassing the safe time to observe a benefit.

Although no significant differences were observed between the groups in most of the analyzed variables, relevant clinical effects were found in the group that received the IR/RED blanket compared with the Sham, RED, and IR group mainly for peak torque and average power at 60°/s, time to exhaustion, VO2max, iLAN, and skin temperature (Table 5). These findings corroborate studies by De Marchi et al. [12] and Albuquerque and Pontes et al. [25] who recommend that the junction of the two wavelengths in the same application provides better results. However, to our knowledge, there are no studies that have compared wavelengths in different groups at the same dosage and application model to improve performance.

Some limitations of the study are relevant mainly for the evaluated population since they are individual athletes, so the

Table 3 Between-group differences at post-LED during the evaluation with the isokinetic dynamometer

Post-sequential application of LED						
Outcomes	Sham vs Red Adjusted mean difference (95% CI)	Sham vs IR Adjusted mean difference (95% CI)	Sham vs Red/IR Adjusted mean difference (95% CI)	Red vs IR Adjusted mean difference (95% CI)	Red vs RED/IR Adjusted mean difference (95% CI)	IR vs Red/IR Adjusted mean difference (95% CI)
I:Q 60°/sec right (%)	1.8 (− 11.1 to 14.8)	− 0,2 (− 13.3 to 13.3)	3.3 (− 9.6 to 16.3)	− 1.9 (− 14.2 to 10.4)	1.4 (− 10.5 to 13.5)	3.36 (− 8.9 to 15.6)
I:Q 60°/sec left (%)	− 4.0 (− 20.1 to 11.9)	− 8.4 (− 25.0 to 8.2)	− 2.0 (− 18.4 to 14.24)	− 4.13 (− 19.9 to 11.3)	2.0 (− 13.3 to 17.3)	6.3 (− 9.2 to 21.8)
I:Q 270°/sec right (%)	− 20.1 (− 72.7 to 32.5)	− 19.2 (− 73.0 to 34.5)	− 2.4 (− 55.2 to 50.4)	0.8 (− 50.6 to 52.3)	17.6 (− 32.6 to 68.0)	16.8 (− 35.2 to 68.9)
I:Q 270°/sec left (%)	2.4 (− 5.7 to 10.6)	1.7 (− 6.6 to 10.0)	− 3.6 (− 12.0 to 4.8)	− 0.7 (− 8.7 to 7.2)	− 6.0 (− 14.1 to 2.0)	− 5.3 (− 13.5 to 2.8)
Torque peak 60°/sec right (N/m)	− 3.8 (− 23.4 to 15.7)	2.8 (− 17.0 to 22.8)	− 6.9 (− 26.7 to 12.8)	6.7 (− 12.3 to 25.8)	− 3.1 (− 22.2 to 16.0)	− 9.8 (− 29.1 to 9.4)
Torque peak 60°/sec left (N/m)	− 5.9 (− 22.3 to 10.3)	1.0 (− 15.5 to 14.7)	− 5.1 (− 21.6 to 11.4)	7.0 (− 9.0 to 23.22)	0.89 (− 15.4 to 17.2)	− 6.19 (− 22.1 to 9.7)
Torque peak 270°/sec right (N/m)	0.1 (− 13.9 to 14.1)	3.3 (− 11.0 to 17.7)	− 50.6 (− 68.0 to − 32.9)*	3.2 (− 10.4 to 16.9)	− 50.6 (− 67.2 to − 34.0)*	− 53.9 (− 70.3 to − 37.4)*
Torque peak 270°/sec left (N/m)	− 3.8 (− 18.5 to 10.9)	2.2 (− 12.7 to 17.2)	− 38.2 (− 55.4 to − 21.0)*	6.1 (− 8.2 to 20.5)	− 34.4 (− 50.3 to − 18.4)	− 40.5 (− 57.3 to − 23.6)*
Average power 60°/sec right (W)	− 4.3 (− 32.5 to 23.5)	5.5 (− 23.0 to 34.1)	− 19.6 (− 47.7 to 8.4)	10.0 (− 17.3 to 37.4)	− 15.1 (− 41.9 to 11.6)	− 25.1 (− 52.5 to 2.1)
Average power 60°/sec left (W)	− 4.1 (− 26.3 to 14.9)	3.6 (− 19.0 to 26.3)	− 8.36 (− 30.6 to 13.9)	7.8 (− 14.0 to 29.7)	− 4.1 (− 25.7 to 17.3)	− 12.0 (− 33.5 to 9.5)
Average power 270°/sec right (W)	− 13.0 (− 67.7 to 41.6)	11.9 (− 43.9 to 67.7)	− 68.4 (− 135.2 to − 1.6)*	24.9 (− 28.4 to 78.3)	− 55.4 (− 120.4 to 9.5)	− 80.3 (− 144.5 to − 16.2)*
Average power 270°/sec left (W)	− 3.1 (− 34.8 to 28.6)	14.7 (− 17.6 to 47.0)	− 66.4 (− 103.8 to − 29.0)*	17.8 (− 13.1 to 48.7)	− 63.3 (− 100.8 to − 25.7)*	− 81.1 (− 118.1 to − 44.0)*

*$p < 0.05$; I:Q, agonist-antagonist relationship; 60°/sec and 270°/sec, angular speed used; right lower limb; left lower limb

Table 4 Between-group differences at 1 week of follow-up during the evaluation with the isokinetic dynamometer

1-week follow-up						
Outcomes	Sham vs Red Adjusted mean difference (95% CI)	Sham vs IR Adjusted mean difference (95% CI)	Sham vs Red/IR Adjusted mean difference (95% CI)	Red vs IR Adjusted mean difference (95% CI)	Red vs RED/IR Adjusted mean difference (95% CI)	IR vs Red/IR Adjusted mean difference (95% CI)
I:Q 60°/sec right (%)	− 4.3 (− 22.9 to 14.2)	− 6.5 (− 24.0 to 10.9)	− 3.8 (− 20.42 to 14.76)	− 2.2 (− 20.7 to 16.3)	0.5 (− 17.0 to 18.1)	2.7 (− 13.5 to 18.9)
I:Q 60°/sec left (%)	− 3.1 (− 24.7 to 18.5)	4.7 (− 15.8 to 25.2)	− 0.2 (− 19.1 to 18.5)	7.8 (− 13.7 to 29.5)	2.8 (− 17.6 to 23.3)	− 5.0 (− 24.3 to 14.3)
I:Q 270°/sec right (%)	− 3.7 (− 19.0 to 11.6)	− 1.4 (− 16.4 to 13.6)	− 0.6 (− 14.0 to 12.7)	2.3 (− 13.4 to 18.1)	3.0 (− 11.4 to 17.4)	0.7 (− 13.24 to 14.7)
I:Q 270°/sec left (%)	− 5.3 (− 16.4 to 5.6)	− 0.7 (− 11.0 to 9.5)	− 6.6 (− 16.3 to 3.0)	4.6 (− 6.4 to 15.6)	− 1.3 (− 11.8 to 9.1)	− 5.9 (− 15.6 to 6.4)
Torque peak 60°/sec right (N/m)	− 0.5 (− 30.2 to 29.1)	− 11.0 (− 38.8 to 16.7)	− 2.2 (− 28.5 to 24.1)	− 10.5 (− 40.2 to 19.2)	− 1.64 (− 29.9 to 26.6)	8.8 (− 17.5 to 35.3)
Torque peak 60°/sec left (N/m)	3.7 (− 21.4 to 28.9)	− 4.5 (− 28.0 to 19.0)	− 0.3 (− 22.6 to 21.8)	− 8.2 (− 33.4 to 17.0)	− 4.0 (− 28.1 to 20.0)	4.1 (− 18.0 to 26.3)
Torque peak 270°/sec right (N/m)	− 4.6 (− 18.7 to 9.4)	− 6.2 (− 19.5 to 7.0)	− 5.8 (− 18.0 to 6.3)	− 1.6 (− 15.6 to 12.3)	− 1.2 (− 14.6 to 12.1)	0.4 (− 12.1 to 13.0)
Torque peak 270°/sec left (N/m)	4.5 (− 9.3 to 18.4)	− 0.8 (− 13.8 to 12.0)	− 2.5 (− 14.5 to 9.5)	− 5.4 (− 19.1 to 8.3)	− 7.0 (− 20.2 to 6.2)	− 1.6 (− 13.8 to 10.6)
Average power 60°/sec right (W)	1.6 (− 20.7 to 24.1)	− 17.3 (− 38.3 to 3.6)	− 3.6 (− 23.5 to 16.1)	− 18.9 (− 41.7 to 3.7)	− 5.32 (− 26.4 to 15.8)	13.6 (− 6.6 to 33.9)
Average power 60°/sec left (W)	− 1.8 (− 25.3 to 21.7)	− 12.6 (− 34.6 to 9.4)	− 2.7 (− 23.4 to 18.0)	− 10.7 (− 34.3 to 12.8)	− 0.8 (− 23.1 to 21.3)	9.8 (− 10.9 to 30.6)
Average power 270°/sec right (W)	7.9 (− 39.5 to 55.4)	17.4 (− 26.7 to 61.7)	− 16.9 (− 58.2 to 24.3)	9.5 (− 38.5 to 57.6)	− 24.8 (− 69.8 to 20.0)	− 34.4 (− 75.8 to 6.9)
Average power 270°/sec left (W)	9.4 (− 23.4 to 42.4)	− 0.0 (− 30.7 to 30.7)	− 17.1 (− 45.9 to 11.7)	− 9.4 (− 42.9 to 24.0)	− 26.5 (− 57.4 to 4.2)	− 17.1 (− 46.5 to 12.3)

I:Q, agonist-antagonist relationship; 60°/sec and 270°/sec, angular speed used; right lower limb; left lower limb

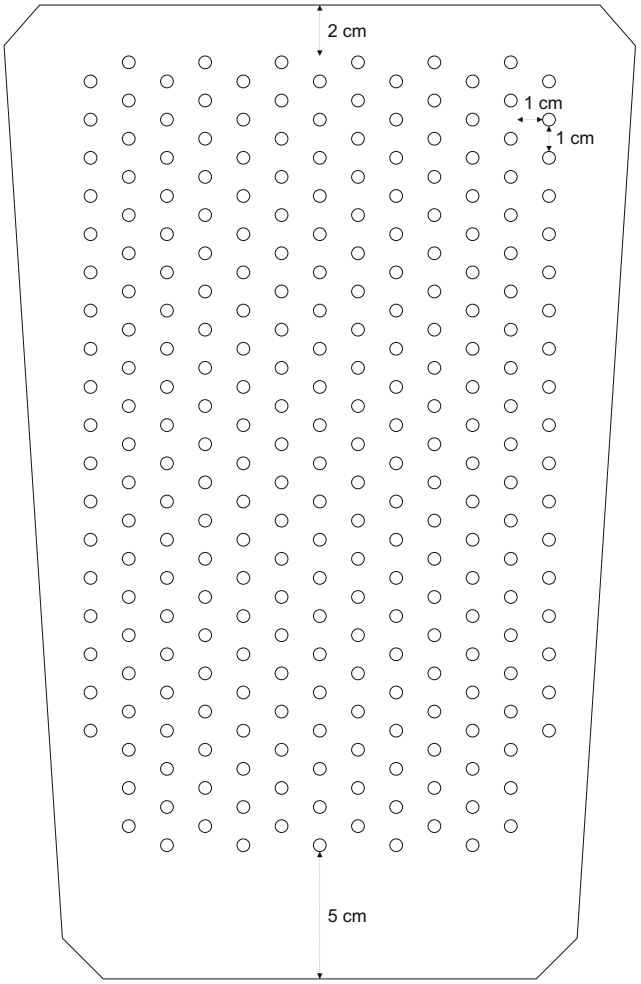

Fig. 3 Schematic of the LED device developed at the institution

volume of training before the beginning of the study between one athlete and another may vary and may become a bias for the athletes. Only male athletes participate in the study, so the results cannot be extrapolated to female athletes, mainly due to the influence of ovarian cycles. It should also be taken into account that the entire study was done in a laboratory environment, without any external influences, so the results may be different when applied to a training and competition environment.

As future perspectives, this study shows that sequenced application of photobiomodulation not associated with training is not the ideal scenario for the performance improvement. Furthermore, the use of two wavelengths, i.e., red and infrared, seems to provide better results. More studies should be done aiming at the methodology and application parameters da PBMT, within a competition or training environment of athletes of different modalities, so that these gaps can be filled and promote the improvement of the clinical quality of photobiomodulation application.

Conclusion

Photobiomodulation, not associated with training, was not able to produce a cumulative effect and increase the strength, endurance, and subjective perception of effort in cycling athletes. However, clinical effects were observed in the association of two wavelengths in the same application, and this seemed to present better results when compared with the red LED and the infrared LED alone, as well as the sham treatment. More studies are needed to determine the better

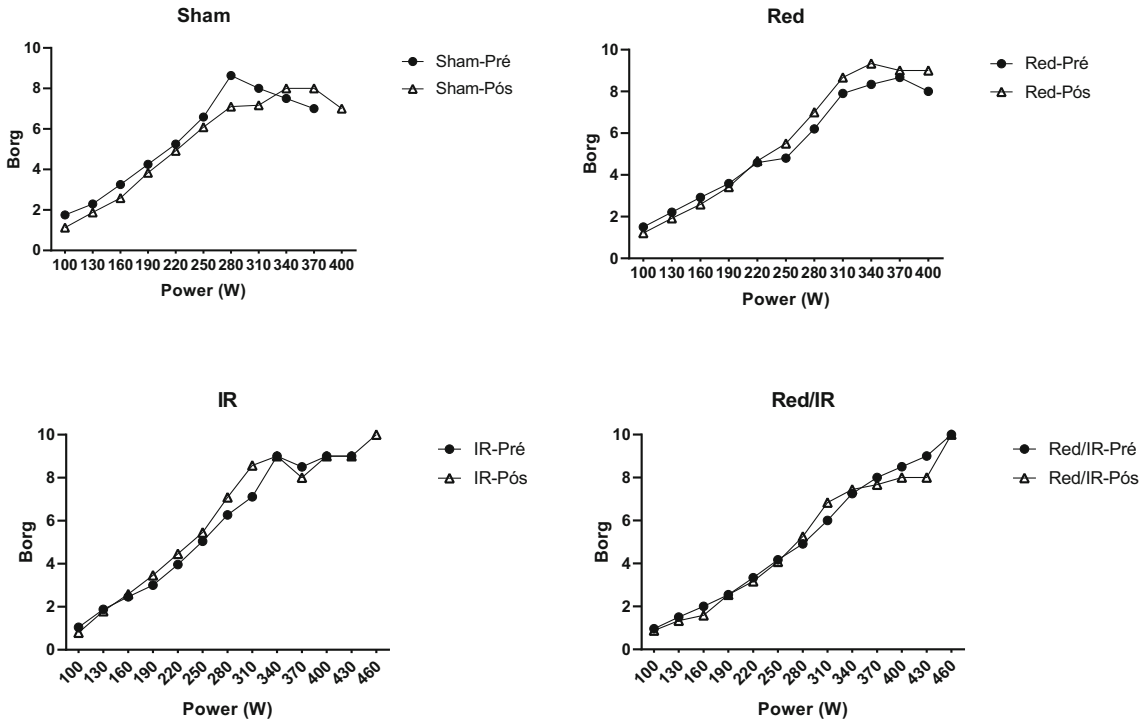

Fig. 4 Values before and after the power LED and subjective perception of individual effort during the incremental test

Table 5 Between-group differences at post-sequential application of LED from total time to exhaustion, VO2max and iVO2max and LAN during the incremental test, and cutaneous temperature of the lower limbs after the incremental test

Post-sequential application of LED						
Outcomes	Sham vs Red Adjusted mean difference (95% CI)	Sham vs IR Adjusted mean difference (95% CI)	Sham vs Red/IR Adjusted mean difference (95% CI)	Red vs IR Adjusted mean difference (95% CI)	Red vs RED/IR Adjusted mean difference (95% CI)	IR vs Red/IR Adjusted mean difference (95% CI)
Total time to exhaustion (s)	46.7 (− 100.8 to 194.37)	66.7 (− 82.9 to 216.3)	− 26.9 (− 180.7 to 126.8)	19.9 (− 126.2 to 166.1)	− 73.7 (− 221.8 to 74.3)	− 93.6 (− 240.2 to 52.9)
VO2máx (l min)	− 0.8 (− 1.7 to 0.0)	− 0.4 (− 1.4 to 0.4)	− 0.5 (− 1.4 to 0.3)	0.3 (− 0.5 to 1.2)	0.2 (− 0.5 to 1.0)	− 0.1 (− 0.9 to 0.7)
iVO2máx(l/kg min)	7.5 (− 29.6 to 44.8)	8.0 (− 30.8 to 46.8)	3.9 (− 34.1 to 42.0)	0.4 (− 35.6 to 36.4)	− 3.58 (− 38.4 to 31.2)	− 4.0 (− 39.2 to 31.1)
LAN1 (W)	12.2 (− 21.0 to 45.6)	13.0 (− 22.0 to 48.1)	3.2 (− 30.7 to 37.3)	0.7 (− 30.9 to 32.4)	− 9.0 (− 39.4 to 21.4)	− 9.7 (− 40.6 to 21.1)
Temperature right limb (°C)	− 0.5 (− 2.0 to 0.8)	0.1 (− 1.3 to 1.5)	− 0.1 (− 1.6 to 1.4)	0.6 (− 0.7 to 2.1)	0.4 (− 0.9 to 1.8)	− 0.2 (− 1.8 to 1.3)
Temperature left limb (°C)	− 0.7 (− 2.1 to 0.6)	0.1 (− 1.2 to 1.5)	− 0.3 (− 1.5 to 1.4)	0.8 (− 0.5 to 2.2)	0.6 (− 0.6 to 2.0)	− 0.1 (− 1.7 to 1.3)

VO2max, maximum oxygen consumption; *iVO2max*, intensity corresponding to VO2max; *LAN1*, first anaerobic threshold

parameters and the time course between application and the photobiomodulation effect.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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