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ORIGINAL ARTICLE

Photobiomodulation does not improve anaerobic performance in well-trained cyclists

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Abstract
To determine if photobiomodulation (PBM) has ergogenic effects on the anaerobic performance of well-trained cyclists. Fifteen healthy male road or mountain bike cyclists participated in this randomized, double-blinded, placebo-controlled, crossover study. Athletes were randomly assigned to receive photobiomodulation (630 nm, 4.6 J/cm^2, 6 J per point, 16 points, PBM session) or placebo intervention (PLA session) in the first session. The athletes then performed a 30-s Wingate test to determine mean and peak average power, relative power, mean and peak velocity, mean and peak RPM, fatigue index, total distance, time to peak power, explosive strength, and power drop. After 48 h, athletes returned to the laboratory for the crossover intervention. The repeated-measures ANOVA test followed by Bonferroni post hoc test or Friedman test with Dunn's post hoc test ($p < 0.05$), and Cohen's d statistic were used for comparisons. Performance in the Wingate test was not significantly different ($p > 0.05$) between PBM and PLA sessions for any variable. Only a small effect size was detected for time to peak power (-0.40; 1.11 to 0.31) and explosive strength (0.38; -0.34 to 1.09). We conclude that irradiation with red light, under a low energy density, does not promote ergogenic effects on the anaerobic performance of cycling athletes.

Keywords Physical fitness · Low-Level Light Therapy · Performance-enhancing effects · Muscle Strength

Introduction

Cycling is an Olympic sport (track, road, mountain bike, and BMX categories) with endurance or anaerobic characteristics, depending on the modality, with an important contribution of anaerobic metabolism in explosive strength and power generation during sprinting and climbing [1–5]. Anaerobic tests demonstrated a strong association of maximal and mean average power with racing performance in cycling athletes [2, 5, 6]. Although road cycling is a predominantly aerobic sport [7], cycling athletes perform several sprints in the course of an endurance race, resulting in elevated levels of anaerobic power production in some moments of the race [8]. In both short (1000 to 4000 m) and endurance trials, a greater anaerobic contribution was required to achieve a higher power output at the onset of the race [2, 8–10]. In cross-country mountain bike cycling races, approximately 18 ± 4 repeated bouts of peak power (559 ± 46 W), 40 ± 14 s, were observed during each lap [8]. However, the authors observed that peak output was significantly higher in the first lap, decreasing in the last two laps [8], suggesting some degree of accumulated fatigue during

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the race. To reach the finish line in the top 5, road cyclists produce a higher peak power (1248 ± 122 W) and average power output of 1020 ± 77 W during the last 13.2 ± 2.3 s sprints [10]. Thus, maintaining the capacity to generate power and delay fatigue may be essential in short and long distances races. Considering the importance of sprinting on cycling performance, even in long-distance races, the use of ergogenic resources that can contribute to maintaining or even improving anaerobic performance within cycling events may be advantageous.

Some ergogenic strategies to improve anaerobic power performance have been used in cyclists, such as nutritional supplementation with caffeine [11], β-alanine [12, 13], creatine [14], and beetroot juice [15]. However, contradictory results in the literature demonstrate no effects of nutritional supplements in improving anaerobic performance and fatigue [15, 16], an ergogenic effect attributed to the placebo effect [11], effects that depend on the type of intermittent efforts and recovery intervals [12, 14], or genetic responsiveness to ergogenic supplements [17]. This interindividual variability and the placebo effects after using ergogenic supplements [16–19] make it difficult to evaluate any ergogenic effects on the anaerobic performance of cyclists. Other ergogenic strategies, such as blood flow restriction and systemic hypoxia [20], balenine supplementation [21], consumption of dark chocolate [22], and electric muscle stimulation [23] also failed to improve sprinting performance. Another concern is the training status of individuals. Some ergogenic aids seemed to improve anaerobic performance in athletes and non-trained subjects [13], whereas others improved anaerobic performance only in non-trained or physically active subjects, without significant effects in well-trained and athlete subjects [24, 25]. In this regard, there is no consensus on the usefulness of ergogenic resources for improving sprinting performance in cycling athletes.

Photobiomodulation (PBM) with low-level light irradiation has ergogenic effects in cyclists, increasing time-to-exhaustion and the recruitment of motor units, and decreasing O_2 deficit during a cycle ergometer fatiguing test at maximum power output [26, 27]. A recent work of our research group demonstrated that photobiomodulation has a potential ergogenic effect on anaerobic performance during the Wingate cycling test in healthy subjects [28]. Irradiation at the red light wavelength (630 nm), with a low dose density (4.6 J/cm^2, 96 J of total energy per lower limb) improved cycling performance in the anaerobic Wingate test (WT), increasing the peak power and velocity (W_{peak}, W/kg, RPM_{max}, V_{peak}), as well as average measurements (W_{mean}, W/kg $_{mean}$, RPM $_{mean}$, V $_{mean}$) in the 30-s Wingate test. PBM may improve anaerobic performance by improving O_2 kinetics and ATP-phosphocreatine synthesis, increasing nitric oxide bioavailability, and recruitment of type II muscle fibers [29–31]. Considering the promising effects of PBM on fatigue in well-trained athletes and anaerobic performance in healthy subjects, the ergogenic effects of PBM may be of interest in improving or maintaining the anaerobic performance of well-trained cyclists during sprint efforts.

Analyzing the possible ergogenic effect of PBM in well-trained cyclists during high-intensity and short-duration sprint exercises is important to establish the usefulness of this ergogenic resource in cycling modalities that require the generation of power and sprints. The study hypothesis was that PBM would increase peak and average power and decrease fatigue during the anaerobic test. Thus, the present study aims to determine if PBM has ergogenic effects on power generation and the fatigue index in well-trained cyclists in the Wingate test.

Methods

Participants

Fifteen healthy male well-trained cyclists (28.33 ± 9.58 years of age, body mass index between 18.5 and 24.9 kg/m^2), who were familiarized with sprint cycling training on a cycle ergometer were enrolled in the study. The volunteers were previously familiarized with the Wingate test before the study. Participants were classified as well-trained and/or competitive cyclists (performance level 3 or more) of road cycling, and or mountain bike cycling, according to the Pauw et al. criteria [32]. Athletes had performed at least 10 h of moderate to intense cycling training per week, for more than 12 months, and had participated in official competitions at the national level in the previous 12 months. Individuals who reported musculoskeletal lesions diagnosed in the previous 6 months, and individuals with previously diagnosed cardiac and metabolic diseases (hypertension, diabetes, autoimmune diseases) were excluded from the study. Only volunteers with skin color according to Fitzpatrick skin types I to IV [33] were included in the sample because the red light (630 nm) wavelength employed in the study is absorbed by the melanin [34] of the epidermis, which could reduce the amount of energy supplied to the muscle.

The experimental procedures were approved by the Research Ethics Committee involving Human Subjects of the State University of Londrina, Londrina-Brazil (protocol no. 2.238.417) and conducted following the Helsinki Declaration. All the volunteers were previously oriented about the research objectives and signed the informed consent form.

Experimental design

The study is a crossover, randomized, double-blind, and placebo-controlled trial. The volunteers were instructed not to perform intense or strenuous physical exercises for 48 h before the tests. They were also instructed not to drink caffeinated beverages and/or foods, ergogenic supplements, and anti-inflammatory medications for 48 h before and during the physical tests. The 30-s Wingate test was chosen because it is designed to evaluate anaerobic performance and can be used to test the effect of ergogenic methods [28, 35–39]. The Wingate test also presents high reproducibility, and reliability when performed in the same cycloergometer if athletes were familiarized with the equipment and test [37, 40, 41]. The Wingate test is considered the gold standard laboratory test for evaluating anaerobic performance and simulates the typical movements of cycling [35–39].

Data collection was performed in the Laboratory of Sports Performance Assessment (LADESP) of the State University of West Paraná, Campus Marechal Cândico Rondon, Paraná, Brazil. On the first day of Wingate tests, athletes were randomized to the photobiomodulation (PBM session) or placebo intervention (PLA session). After receiving the PBM/placebo interventions the athletes performed a standardized warm-up for the Wingate test (Fig. 1), cycling for five minutes without load, at a rate of 60 RPM, with two sprints of five seconds (in the second and third minutes) [42]. After a 60-s rest, the volunteers performed the Wingate test. After 48 h, in the second session, the volunteers were submitted to the treatment not applied in the first session (crossover), and the same data collection procedures were applied (Fig. 1). A 48-h interval is enough time to avoid the residual effect of photobiomodulation treatment (carry-over effect) between Wingate test sessions [28]. The athletes were used to perform the 30-s Wingate tests in the cycloergometer used in the present study, as part of their routine performance tests for planning their training periodization during competitive seasons.

The subjects were randomly assigned to receive either PBM or PLA interventions by drawing sealed opaque envelopes. The treatment and the performance analysis were performed by two researchers who did not take part in the data collection and analysis. The Wingate test was applied by a third researcher who did not know the study interventions. All tests were performed by participants at the same hour each day, by the same researchers.

Photobiomodulation

Photobiomodulation was applied immediately before the warm-up procedures (Fig. 1). The equipment was maintained in direct contact with the skin and perpendicular to the anterior and posterior surfaces of the thighs and calves of the volunteer, with the participant resting on a stretcher using protective swimming goggles with opaque lenses for blocking light irradiation and headphones so that the volunteer did not notice any light and sound signal emitted by the equipment. Eight points of the anterior region (four in the rectus femoris, 2 in the *vastus medialis* and 2 in the *vastus lateralis*), four points in the posterior region (two in the femoral biceps and two in the semitendinosus), and four points in the sural triceps were irradiated (Fig. 2). A commercial light-emitting diode probe (Bios Therapy II, Bios EquipamentosMédicos, São José dos Campos, São Paulo, Brazil) was used, according to the manufacturer's instructions for this intervention. The PBM treatment specifications are shown in Table 1.

Fig. 2 Application points (black circles) of the photobiomodulation

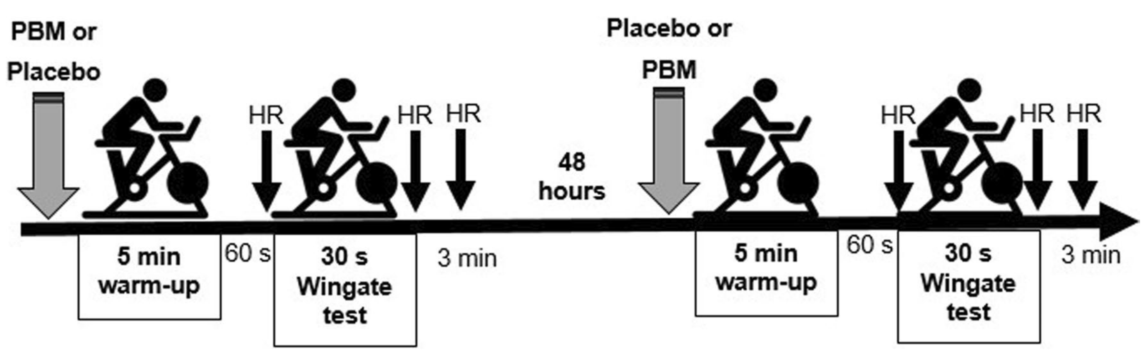

Fig. 1 Experimental design. HR: heart rate

Table 1 Photobiomodulation dosimetry

Specifications	
Wavelength	630 nm
Frequency	Continuous
Optical output	300 mW
Spot area	1.32 cm^2
Power density	230 mW/cm^2
Energy per point	6 J
Total energy per lower limb	96 J
Number of irradiated points	16
Irradiated area	21.12 cm^2
Energy density	4.6 J/cm^2
Time per point	30 s
Time of treatment	320 s
Application mode	Stationary in contact with the skin surface

The PLA intervention was performed with the equipment turned off and positioned at the same points and for the same time as the PBM treatment.

Wingate test

The Wingate test was performed in a Biotec 2100 Cyclometer (Cefise Biotecnologia Esportiva, Nova Odessa, São Paulo, Brazil), in which the participants cycled for five minutes without load, at a rate of 60 revolutions per minute (RPM), with two sprints of five seconds (in the 2nd and 3rd minutes) for warm-up. After the warm-up, the participants rested for one minute on the cycle ergometer, and a load of 10% of their total body mass was individually adjusted for the subsequent test. Participants then cycled at full speed for 30 s under standardized verbal motivation [35]. The peak power (W_{peak}) and average power (W_{mean}), relative power (W/kg), maximum relative power (W/kg_{peak}), average relative power (W/kg_{mean}), peak velocity (Km/h, V_{peak}), mean velocity (V_{mean}), peak revolution per minute (RPM_{peak}), maximum RPM (RPM_{max}) and average RPM (RPM_{mean}), fatigue index (%F), and total distance covered (TD) were quantified during the Wingate test. The data were recorded at a second-by-second frequency, using Ergometric 6.0.6 software (CefiseBiotecnologiaEsportiva, Nova Odessa, São Paulo, Brazil). Time to Peak Power (seconds, tW_{peak}), Explosive Strength (the tW_{peak} divided by $W/kg_{paek,}$ ExS), and Power Drop (PD) were also determined. The reliability and reproducibility of the measures used, such as power ratings and anaerobic capacity, have already been determined in the literature [35, 37].

Heart rate

The resting heart rate (HR Pre) was measured in subjects in the cycling position during the five final seconds of recovery after the warm-up, before the Wingate test. The HR was monitored during the test sessions and three minutes after the test to record HR peak during exercise and HR recovery. HR was monitored using a portable heart rate monitor (V800, Polar, Kampele, Finland).

Statistical analysis

The sample size was estimated based on a previous study [28] and for peak power performance, a minimum of seven subjects was necessary, considering an effect size of 1.11, to achieve an 80% statistical power with a type I error limited to 5%. The normality of the data was tested with the Shapiro–Wilk test. As variables presented normal distribution, the results are expressed as mean and standard deviation. The comparisons of the parametric data were performed with the paired t-test (considered measures of the same subjects twice, in a crossover design using PBM or placebo treatments). The effect size (ES) for the comparison of means was calculated using Cohen's d statistic and classified as small (ES > 0.2), moderate (ES > 0.5), and large (ES > 0.8) [43]. The HR data and the carry-over effect were analyzed by the ANOVA one-way and post hoc Bonferroni tests. Statistically significant differences were set at $p < 0.05$.

Results

Performances in the Wingate test are shown in Table 2. There were no significant differences in Wingate test performance between PBM and PLA sessions (Table 2). Only a small effect size was detected for time to peak power and explosive strength (Table 2).

HR was not statistically different between PLA and PBM sessions (Table 2).

Discussion

Contrary to the study hypothesis, PBM did not improve Wingate test performance in well-trained cyclists. The main findings of the study demonstrated that PBM, under the same energy doses used in non-athletes [28] did not improve anaerobic performance in well-trained cyclists. No significant differences were observed between the performance indices related to alactic (10 s) and lactic metabolism [35].

Cycling events in different modalities finish with a sprint or a short acceleration at the end of the trial [2, 10]. As a result, there is growing interest in understanding and

Table 2 Wingate test performance and fatigue index ($n=15$)

	PLA	PBM	*P*	Effect size: 95% CI
Peak Power (W)	1195.9 ± 180.9	1194.4 ± 186.5	0.93	-0.01 (trivial): -0.71 to 0.72
Average Power (W)	859.2 ± 103.4	858.8 ± 108.6	0.95	0.00 (trivial): -0.71 to 0.72
Peak Relative power (W/Kg)	14.9 ± 1.8	14.9 ± 1.9	0.96	0.00 (trivial): -0.71 to 0.72
Average Relative Power (W/Kg)	10.7 ± 1.2	10.7 ± 1	0.82	0.00 (trivial): -0.71 to 0.72
Peak RPM	144.8 ± 17.4	145.6 ± 18.9	0.73	0.04 (trivial): -0.76 to 0.67
Average RPM	104.4 ± 9.6	105 ± 12	0.59	0.06 (trivial): -0.77 to 0.66
Peak Velocity (Km/h)	45.6 ± 5.5	45.4 ± 5.8	0.87	-0.04 (trivial): -0.68 to 0.75
Average Velocity (Km/h)	32.9 ± 3	32.8 ± 3.7	0.8	-0.02 (trivial): -0.75 to 0.69
Total Distance (m)	273.6 ± 25.2	275.8 ± 31.5	0.49	0.08 (trivial): -0.64 to 0.79
Fatigue Index (%)	49.8 ± 10	51.7 ± 12.2	0.51	0.17 (trivial): -0.64 to 0.79
Time to Peak Power (s)	4.1 [3-5]	3.7 [3-5]	0.11	-0.40 (small): -1.11 to 0.31
Explosive Strength (W/s)	300 ± 76.3	329.3 ± 78.3	0.2	0.38 (small): -0.35 to 1.09
HR Pre	111.9 ± 8.4	112.1 ± 8.2	0.69	-0.02 (trivial): -0.74 to 0.70
HR max	183.7 ± 10.6	182.9 ± 9.8	0.15	0.07 (trivial): -0.67 to 0.81
HR Post-3-min	138.1 ± 7.4	137.7 ± 7.8	0.59	-0.05 (unclear): -0.78 to 0.68

applying strategies to improve sprint capacity, either by increasing peak power and relative power or maintaining power over time [11, 23, 26, 44]. The Wingate test is an anaerobic test and is a validated tool to assess improvements or maintenance of power and anaerobic capacity in sprints [6, 35, 37]. The main indices obtained in the test are the peak power, which is directly related to the alactic anaerobic metabolism and the use of ATP-CP, the average power, which is an index of the lactic anaerobic metabolism and the use of muscle glycogen, and the fatigue index [35, 37]. Considering the cycling movements performed in the Wingate test, it could be a valuable tool to identify the effects of PBM and other ergogenic resources in cycling athletes.

We have previously used the Wingate test to investigate the effects of PBM on sprint efforts (30-s Wingate test) in physically active subjects [28]. The main differences between the previous [28] and present study were the performance of volunteers in the Wingate and their training status. Subjects in Molina Correa et al. [28] study were physically active (perform at least 150 min of moderate to intense physical activity per week but did not practice any systematic training program or sport modality), were male young adults, and had body mass index close to the athletes investigated in the present study[28]. Whereas, the present study investigated male young and well-trained cyclists, classified as level 4 to 5 training status, which means they trained more than 10 h/week or covered more than 250 km/week [32] and were participating in national official competitions in the last year. Although the Molina Correa et al. [28] study demonstrated increased performance in peak and mean indices, suggesting improved alactic and lactic metabolism, none of these ergogenic effects were found in trained cyclists. These results suggest that PBM may be not efficient for improving performance in trained subjects, or that PBM may elicit different effects depending on training status. A recent systematic review concluded that the ergogenic effect of PBM may be useful to improve endurance and time to exhaustion in cycling exercises, but it does not work in many other modalities involving endurance and complex movements [45]. The literature points out many reasons why PBM may not have ergogenic effects in all situations, including cell mitochondrial density [46], light wavelength [46–48], energy dose (power density, total energy, irradiated area, and output frequency) [49–53], training status, and individual responsiveness [54, 55].

Physiologically, subjects unfamiliar with maximum efforts may present different responses to ergogenic resources since they unleash different metabolic responses, a different pattern of neural drive, and different muscle fiber types and recruitment patterns compared to athletes [56, 57]. In the study of Molina et al., (2020), the authors investigated non-athletes and physically active subjects and suggested that PBM could increase Wingate test performance, stimulating phosphocreatine resynthesis and increasing muscle oxygenation and recruitment of fast type II muscle fibers due to the release of nitric oxide (NO). In this sense, photobiomodulation may have had less evident effects on well-trained cyclists because they are adapted to greater recruitment of fast-twitch fibers to achieve high performance (Faria et al., 2005).

The low-level light irradiation of living tissues with red to near-infrared light can increase ATP synthesis and NO release, acting on cytochrome c oxidase, an enzyme that reduces oxygen at the end of the mitochondrial respiratory chain [29, 58, 59]. Whereas improved ATP synthesis and NO release may improve endurance performance [24, 26, 30, 60, 61], NO release can act on anaerobic performance, increasing the recruitment of fast-twitch fibers [62, 63].

Studies employing strategies that stimulate NO release, such as supplementation with NO inducers, have demonstrated contradictory results in well-trained cyclists and recreationally trained men [36, 64–67]. A study supplemented well-trained cyclists with Montmorency tart cherry juice, an extract rich in NO-inducing polyphenols, and did not find increased tissue oxygenation and reduced fatigue during a 60-s all-out cycle sprint, despite increased mean and peak power in the first 20-s of the test [65]. Another study demonstrated that beetroot juice (a NO-inducer) supplementation did not improve performance in a 1-km time trial in well-trained cyclists [64]. However, in recreationally trained men, supplementation with NO-inducers increased mean and peak power output, and reduced time to peak power and the fatigue index [36, 66, 67]. Other studies also did not find ergogenic effects using arginine supplementation or beetroot juice in successive Wingate tests in physically active men [68, 69]. These results suggest that the release of NO could improve performance in alactic or lactic anaerobic performance depending on the type of NO inducer, physical test, or training status. However, in the case of well-trained cyclists, the NO release induced by PBM may not account for any additional improvement in anaerobic performance.

Future studies could investigate cyclists specialized in sprints, time trials, and climbing, and the behavior of physiological markers (i.e., blood lactate, creatine kinase) before and after effort. Considering the different genetic backgrounds and physiological adaptations, motor unit recruitment patterns, and mitochondrial density [2, 70, 71], PBM may have different ergogenic and recovery effects depending on the cycling modality and training level. Another concern is about energy dose, since the physiological adaptations of trained athletes (increased volume of muscle mass, mitochondrial density, and recruitment of motor units) may require higher doses of energy to reach ergogenic effects. Cycling athletes start from a higher level of anaerobic and aerobic capacity when compared to physically active healthy individuals [2, 72], so the ergogenic effects may be more discrete or absent.

We conclude that the irradiation of red light, under a low dose density (96 J, 6 J/cm^2) but covering the main muscles of the lower limbs, does not promote acute ergogenic effects on anaerobic capacity in cycling athletes.

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Data availability The data that support the findings of this study are available from the corresponding author, [SPR] or first author [LJFF], upon reasonable request.

Declarations

Competing interests The authors report there are no competing interests to declare.

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