



LED session prior incremental step test enhance VO_{2max} in running

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Abstract

This study aimed to investigate the effect of prior LED sessions on the responses of cardiorespiratory parameters during the running incremental step test. Twenty-six healthy, physically active, young men, aged between 20 and 30 years, took part in this study. Participants performed two incremental load tests after placebo (PLA) and light-emitting diode application (LED), and had their gas exchange, heart rate (HR), blood lactate, and rating of perceived exertion (RPE) monitored during all tests. The PLA and LED conditions were compared using the dependent Student *t* test with significance set at 5%. The *T* test showed higher maximum oxygen uptake (VO_{2max}) (PLA = 47.2 ± 5.7 ; LED = 48.0 ± 5.4 ml kg^{-1} min^{-1} , trivial effect size), peak velocity (V_{peak}) (PLA = 13.4 ± 1.2 ; LED = 13.6 ± 1.2 km h^{-1} , trivial effect size), and lower maximum HR (PLA = 195.3 ± 3.4 ; LED = 193.3 ± 3.9 b min^{-1} , moderate effect size) for LED compared to PLA conditions. Furthermore, submaximal values of HR and RPE were lower, and submaximal VO_2 values were higher when LED sessions prior to the incremental step test were applied. A positive response of the previous LED application in the blood lactate disappearance was also demonstrated, especially 13 and 15 min after the test. It is concluded that LED sessions prior to exercise modify cardiorespiratory response by affecting running tolerance during the incremental step test, metabolite clearance, and RPE. Therefore, LED could be used as a prior exercise strategy to modulate oxidative response acutely in targeted muscle and enhance exercise tolerance.

Keywords Phototherapy · Ergogenic · Aerobic metabolism · Cardiorespiratory · Blood lactate

Introduction

The application of light, by sources of low-level laser or light-emitting diode (LED), has been widely studied in health sciences and potentially aids the prevention and recovery of injuries, as well as anti-inflammatory and analgesic effects, although the influence on human exercise, as well as sport-related context, as injuries, soreness, training recovery, rehabilitation, and performance enhancement, should be better explored [1–3]. Moreover, little theoretical bases were found to support LED as ergogenic aid for oxidative phosphorylation metabolism response.

Nevertheless, experimental approaches have already observed structural and functional responses supporting oxidative metabolism enhancement, such as increased mitochondrial density, size and membrane potential, increased oxidative enzyme activities, blood lactate disappearance and phosphocreatine shuttle, improvement of oxidative stress deleterious parameters, and increased microcirculation with hyperemia at the point of light application [2, 4–6].

The studies associating LED or laser sessions to endurance exercise in humans reported both acute and chronic responses, such as increased exercise tolerance, changes in metabolic equivalent, lower submaximal heart rate and double product [7], higher absolute and relative values of maximal oxygen uptake (VO_{2max}) [4, 8], and improvement in fatigue index [9].

Then, LED sessions prior to the incremental step test could highlight the ergogenic effects on muscle metabolic profile, by means of higher economical submaximal pace and maximal metabolic rate. Thus, it was hypothesized that prior LED session is a priming exercise strategy able to modify VO_2 cost acutely, which could be incorporated in the training schedule to improve performance and tolerance during high-intensity running exercise.

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Methods

Twenty-six healthy, Caucasian, physically active, young men, aged between 20 and 30 years, volunteered to take part in this study. Exclusion criteria included any previous lower limb musculoskeletal injury in the previous 3 months; the use of any kind of anti-inflammatory medicine, nutritional supplements, or pharmacological agents with ergogenic effect; and undertaking of systematic running training.

The participants had anthropometric variables measured by a single evaluator with large experience. Body weight and height were measured using standardized procedures, and body fat was determined by the sevenfold equation by Jackson and Pollock [10]. The characteristics of the subjects were (mean \pm SD) age 27.8 ± 1.7 years, body weight 78.6 ± 9.1 kg, height 178.1 ± 5.9 cm, body mass index (BMI) 24.9 ± 2.4 kg m⁻², and body fat $17.5 \pm 4.0\%$. Prior to testing, written informed consent was obtained from all participants. The experimental protocol was approved by the university's ethics committee (no. 147.362/2012).

Participants went to the laboratory (temperature = 20–22 °C and relative humidity = 50–60%) three times on different days, at the same time of the day for familiarization with the motorized treadmill, and underwent two incremental load tests in the experimental conditions: placebo (PLA) and LED application (LED).

The experimental conditions were performed in random order with a double-blinded control and the assistance of a second researcher turning the device on or not for energy emission. During the LED sessions, subjects remained seated, and to avoid the identification of the experimental condition by means of audible or visual signals emitted by the device, the subjects were blindfolded and used a headphone.

The minimum interval between tests were 48 h and the maximum 72 h for a maximum period of 2 weeks. Participants were instructed to follow the same nutritional routine and abstain from alcohol and stimulating drinks 24 h prior to testing, and avoid strenuous exercise during the project period.

The incremental exercise tests were performed on a motorized treadmill (Super ATL, Inbrasport, Porto Alegre, Brazil), with the gradient set at 1%. After a warm-up that consisted of walking at 6 km h⁻¹ for 3 min, the protocol started with an initial speed of 8 km h⁻¹, followed by an increase of 1 km h⁻¹ every 3 min between each successive stage until volitional exhaustion. Consistently across each trial, participants were strongly encouraged, verbally, to perform the maximum effort [11].

Gas exchange was monitored throughout the test with data collected breath by breath (Quark® PFT, COSMED, Italy). VO₂ data were reduced to 15-s average intervals to determine the submaximal VO₂ (VO_{2sub}), and the highest value obtained during the incremental test within these intervals was considered VO_{2max}. The observation of the VO₂ plateau at the end of the incremental test was considered to establish the occurrence of

VO_{2max} [12]. If the phenomenon has not been observed, the maximal effort and VO_{2max} were deemed to be achieved if the incremental test met two of the following criteria: (1) peak lactate concentration (LA_{peak}) higher than 8 mmol l⁻¹, (2) maximal heart rate (HR_{max}) within ± 10 beats min⁻¹ of age-predicted HR_{max} ($206 - 0.7 \cdot \text{age}$) [13], and (3) peak rating of perceived exertion (RPE_{peak}) greater than 18 in the 6–20 Borg scale. The peak pulmonary ventilation (VE_{peak}) and pulmonary ventilation related to the VO_{2max} (V_E·VO_{2max}) were also described based on the collected data reduced to 15-s intervals. In addition to VO_{2max} determination, the first intensity at which VO_{2max} occurred was considered the VO_{2max} velocity (vVO_{2max}) [14].

The peak running speed (V_{peak}) of the incremental test was calculated as the speed of the last complete stage added to the completed fraction of the incomplete stage [15], calculated according to the equation “V_{peak} = V_{complete} + t/T”, in which V_{complete} is the running speed of the last complete stage, t the number of seconds sustained during the incomplete stage, and T is the number of seconds required to complete a stage (i.e., 180 s for the protocol).

The ventilatory threshold (VT) was determined by the ventilatory equivalent method, considering an increase in O₂ equivalent (VE·VO₂⁻¹) with no increase in CO₂ equivalent (VE·VCO₂⁻¹), and by the excess CO₂ method, with the intensity at which a non-linear increase in carbon dioxide production occurred (VCO₂) [16]. The VO₂ related to VT was also determined (VO_{2VT}). The slope of the linear regression between VO₂ (ml min⁻¹) and velocity (m min⁻¹) was also calculated, producing the equivalent VO₂ (eVO₂, ml m⁻¹).

Before each incremental test, the Quark® PFT gas analyzer (COSMED, Italy) was calibrated with ambient air and constant concentration of O₂ (16%) and CO₂ gas (5%), whereas the bi-directional turbine (flow meter) was also calibrated using a 3-l syringe, following all the manufacturer's recommendations.

Before testing, participants were familiarized with the 6–20 Borg scale [17], which was used to measure the rating of perceived exertion (RPE) during the last 10 s of each stage and at exhaustion. The highest RPE value was adopted as the peak RPE (RPE_{peak}). Heart rate (HR) was recorded throughout the tests (Polar RS800sd, Kempele, Finland), and the data was reduced to 15-s intervals to determine submaximal HR (HR_{sub}); maximal HR (HR_{max}) was defined as the highest HR value recorded during the test.

Earlobe capillary blood samples (25 μ l) were collected into a glass microhematocrit capillary tube before and at the end of each test and at the 3rd, 5th, 7th, 9th, 11th, 13th, and 15th minutes after tests, during passive recovery sitting in a comfortable chair. From these samples, LA_{pre}, LA_{0-min}, LA_{3-min}, LA_{5-min}, LA_{7-min}, LA_{9-min}, LA_{11-min}, LA_{13-min}, and LA_{15-min} were subsequently determined by electroenzymatic methods using the YSI 2300 STAT (Ohio, USA) automated analyzer (accuracy $\pm 2\%$). The peak lactate (LA_{peak}) was defined for each subject as the highest value among the passive recovery samples.

The LED application protocol had a total time of 2 min and 30 s (30 s per point, with application in both legs simultaneously); the same procedures were used in the PLA and LED conditions, with respect to the presence or absence of light emission for each condition. The irradiation intervention started 5 min before the incremental load test, in contact mode with the LED cluster held stationary with slight pressure at a 90° angle to the skin at each of the five treatment points [2, 18].

The application was carried out in two points of the quadriceps muscle, two points of the femoral biceps muscle, and one point of the gastrocnemius muscle, along the distribution axis of muscle fibers in both legs [4, 8]. The LED equipment THOR® brand (THOR® brand, England) was used, with two clusters of 104 infrared LED diodes each. The technical parameters are presented in Table 1.

Statistical analysis

Data are presented as mean \pm SD and were analyzed using the Statistical Package for the Social Sciences 15.0 software (SPSS Inc., USA). The Shapiro-Wilk test was used to check the normality of the data distribution. The PLA and LED conditions were compared using the dependent Student *t* test. Statistical significance was set at $p < 0.05$. Mixed ANOVA for repeated measures was used to compare pre- and post-test lactate concentrations, among the two experimental conditions. Mauchly's test of sphericity was used to determine data normality, and if necessary the Epsilon adjustment Greenhouse-Geisser to determine the main effect. For multiple comparisons, Bonferroni adjustment was used. Bland-Altman analysis was also used to calculate the bias and the 95% limits of agreement. As complementary analysis, the effect size (ES) was calculated to determine the magnitude of change in each condition using the following equation:

$$ES = (M1 - M2) \div ((SD1 + SD2) \div 2)$$

Table 1 Parameters for the LED application

Number of diodes	104
Wavelength	850 nm (infrared)
Frequency	0–1500 Hz
Power output	30 mW
LED area	0.2 cm ² per diode
Power density	150 mW cm ⁻²
Energy irradiated by each diode	0.9 J
Total energy irradiated	93.6 J per cluster
Energy density	4.5 J cm ⁻²
Exposure time	30 s at each point

Note that M1 and M2 are the average of each condition, and SD1 and SD2 are the respective standard deviations. The ES was classified according to Cohen [19] as ≤ 0.20 (trivial), between 0.21 and 0.50 (small), between 0.51 and 0.80 (moderate), and > 0.80 (large).

Results

The comparison between PLA and LED conditions for the variables obtained during the maximum incremental test are presented in Table 2. The LED condition presented effect, especially on maximum variables, when compared to the PLA condition, with higher effect sizes found for HR_{max} (moderate ES), VO_{2VT}, and RPE_{peak} (small ES). No effect of LED application was found for intensities related with VO₂ response during the incremental running test, while vVO_{2max} occurred at $97.42 \pm 1.94\%$ of V_{peak} and $96.90 \pm 3.43\%$ of V_{peak} respectively for LED and PLA condition.

Figure 1 shows the scattering curve of VO₂ in PLA and LED condition. Since the variance of LED explains 90% of the variance of PLA, it can be stated that the prior LED session ensures the validity of the test response, even requiring different VO₂ values. The bias (0.74) and 95% limits of agreement (-2.77 ; 4.24) also ensure a high agreement between conditions.

Table 3 shows the blood lactate concentrations before and after the maximum incremental tests. Mixed ANOVA highlighted the effect of sampling moments ($p < 0.001$) and experimental condition ($p < 0.001$). Between the 13th and 15th minutes after the test, the blood lactate concentrations were statistically lower for the LED compared to PLA condition; moreover, from the 9th minute the ES among the conditions went from trivial to small, reinforcing lower concentrations for the LED group until the 15th minute. However, the pattern of differences among all sampling moments was similar for LED and PLA conditions. For LED condition, 77% of the subjects reached LA_{peak} after 3 min and only 58% of subjects for PLA condition; after 5 min, 96% of subjects in the LED condition and only 85% in the PLA condition had already reached the LA_{peak}.

Figure 2 shows submaximal values of HR, RPE, and VO₂ variables measured during the incremental stages of the maximum running test. Only completed stages for both conditions were taken into account for these analyses. Twenty-six subjects completed the stage of 11 km h⁻¹ for both conditions, 24 subjects reached 12 km h⁻¹, 16 subjects reached 13 km h⁻¹, 9 subjects reached 14 km h⁻¹, and only four subjects completed 15 km h⁻¹. For all variables, no differences between conditions were found in the first stage (6 km h⁻¹) and in the last stage (15 km h⁻¹) for RPE and VO₂.

Table 2 Comparison and effect size (ES) between the placebo (PLA) and LED (LED) conditions for the variables obtained in maximum incremental load test: total time, maximum oxygen uptake (VO_{2max}), velocity related to the VO_{2max} (vVO_{2max}), peak ventilation and

ventilation related to VO_{2max} (VE_{peak} and $V'Ev'_{O2max}$), peak velocity (V_{peak}), ventilatory threshold (VT), VO_2 related to VT (VO_{2VT}) and maximum heart rate (HR_{max})

Incremental test	PLA ($n = 26$)	LED ($n = 26$)	<i>p</i>	ES
Total time (s)	1338.4 ± 213.3	1362.7 ± 209.1	< 0.001	0.11
V'_{O2max} (ml min ⁻¹)	3722.9 ± 530.1	3808.4 ± 532.3	0.027	0.16
V'_{O2max} (ml·kg min ⁻¹)	47.2 ± 5.7	48.0 ± 5.4	0.045	0.13
vV'_{O2max} (km h ⁻¹)	13.0 ± 1.3	13.2 ± 1.2	0.105	0.16
$V'Ev'_{O2max}$ (l min ⁻¹)	139.0 ± 24.3	142.2 ± 22.0	0.175	0.14
VE_{max} (l min ⁻¹)	147.1 ± 22.7	149.4 ± 21.8	0.087	0.10
V_{peak} (km h ⁻¹)	13.4 ± 1.2	13.6 ± 1.2	< 0.001	0.11
VT (km h ⁻¹)	11.2 ± 0.8	11.3 ± 0.9	0.154	0.12
eV'_{O_2} (ml m ⁻¹)	1015.9 ± 196.6	1043.3 ± 203.5	0.115	0.14
V'_{O2VT} (ml kg ⁻¹ min ⁻¹)	40.1 ± 4.6	41.3 ± 4.3	0.005	0.26
HR_{max} (b min ⁻¹)	195.3 ± 3.4	193.3 ± 3.9	0.018	0.53

Discussion

The aim of this study was to investigate the effect of prior LED session on the responses of cardiorespiratory parameters during the running incremental step test. Despite low effect sizes found between conditions with and without the use of LED, the findings suggested that LED session could be a strategy prior to running exercise, leading to an acute enhancement of maximum and submaximal VO_2 and reduction of HR and RPE, as well as increasing the time limit and final velocity achieved during the incremental step test, without altering the velocity associated to both VO_{2max} and VT.

The biological responses of light application is mainly related to the increased availability of energy due to acute and chronic increased ATP synthesis by lactic and alactic metabolic pathways. Studies demonstrated that mitochondrial function and structure changed positively; the enzymatic activity

of all mitochondrial respiration complexes and enzymes related to aerobic metabolism was increased after application of light [5, 20–23], ensuring a greater rate of ATP synthesis by oxidative metabolism at higher-intensity exercises and as a consequence a delayed activation of type II muscle fibers; therefore, anaerobic ATP resynthesis might occur in the later steps of an incremental test.

Alves et al. [8] conducted incremental tests using a cycle ergometer in the placebo and low-level laser conditions and found a significant effect on the peak values of VO_2 (placebo 31 ± 9 and laser 33 ± 10 ml kg⁻¹ min⁻¹). However, despite the similar respiratory responses compared to the present study, no effect was found on the peak power (placebo 185 ± 51 and laser 182 ± 48 W); according to authors, this could be due to few laser application points, the use of an ergometer with great peripheral request, and a mixed sample of men and women.

On the other hand, De Marchi et al. [4] conducted the first study which aimed to verify the effect of low-level laser in respiratory parameters during the incremental running test in men, and similar to the results of the present study, they found differences when comparing the laser to placebo conditions: higher total time (placebo 697 ± 84 and laser 711 ± 88 s) and relative VO_{2max} (placebo 48 ± 5 and laser 49 ± 5 ml kg⁻¹ min⁻¹). The authors attributed the improvement in respiratory parameters and performance, among other factors, able to enhance aerobic metabolism, to the positive responses of light on oxidative stress, reducing oxidative damage and increasing the concentration of antioxidants [24].

All of these mechanisms may account for the significant responses of LED in VO_{2max} and VO_{2sub} values, despite no effect on exercise velocity associated with VT and vVO_{2max} . Although other studies did not reported values to vVO_{2max} , their submaximal exercise intensity corresponding to aerobic and anaerobic thresholds showed no difference when

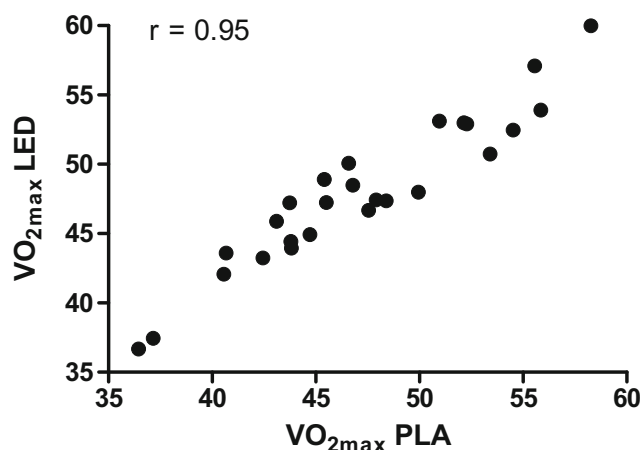


Fig. 1 Scattering curve between maximal oxygen uptake on LED ($VO_{2maxLED}$) and placebo ($VO_{2maxPLA}$) conditions

Table 3 Comparison and effect size (ES) between the placebo (PLA) and LED (LED) conditions for the blood lactate concentrations before (LA_{pre}) and after incremental test (LA_{0-min} , LA_{3-min} , LA_{5-min} , LA_{7-min} , LA_{9-min} , LA_{11-min} , LA_{13-min} , LA_{15-min}), and peak lactate (LA_{peak})

Blood lactate (mmol l^{-1})	PLA ($n = 26$)	LED ($n = 26$)	p	ES
LA_{pre}	1.1 ± 0.6	1.0 ± 0.4	0.200	0.22
LA_{0-min}	$8.8 \pm 1.5a$	$8.5 \pm 1.0a$	0.226	0.24
LA_{3-min}	$9.6 \pm 1.9ab$	$9.6 \pm 1.8ab$	0.684	0.002
LA_{5-min}	$9.5 \pm 1.7a$	$9.2 \pm 1.5a$	0.323	0.17
LA_{7-min}	$9.0 \pm 1.6ad$	$8.8 \pm 1.6ac$	0.231	0.14
LA_{9-min}	$8.7 \pm 1.6acd$	$8.4 \pm 1.4acd$	0.186	0.22
LA_{11-min}	$8.2 \pm 1.9acd$	$7.8 \pm 1.3abcdef$	0.109	0.26
LA_{13-min}	$7.7 \pm 1.9abcdefg$	$7.2 \pm 1.3abcdefg$	0.030	0.31
LA_{15-min}	$7.3 \pm 2.8abcdefgh$	$6.6 \pm 1.3abcdefgh$	0.001	0.30
LA_{peak}	10.0 ± 1.8	9.8 ± 1.7	0.280	0.12

^a $p < 0.05$ related to LA_{pre}

^b $p < 0.05$ related to LA_{0-min}

^c $p < 0.05$ related to LA_{3-min}

^d $p < 0.05$ related to LA_{5-min}

^e $p < 0.05$ related to LA_{7-min}

^f $p < 0.05$ related to LA_{9-min}

^g $p < 0.05$ related to LA_{11-min}

^h $p < 0.05$ related to LA_{13-min}

comparing low-level laser condition to the placebo during the incremental step test on treadmill [4] or cycle ergometer [8].

These reports suggest that the prior light sessions influence modifications in gas exchange and biochemical and

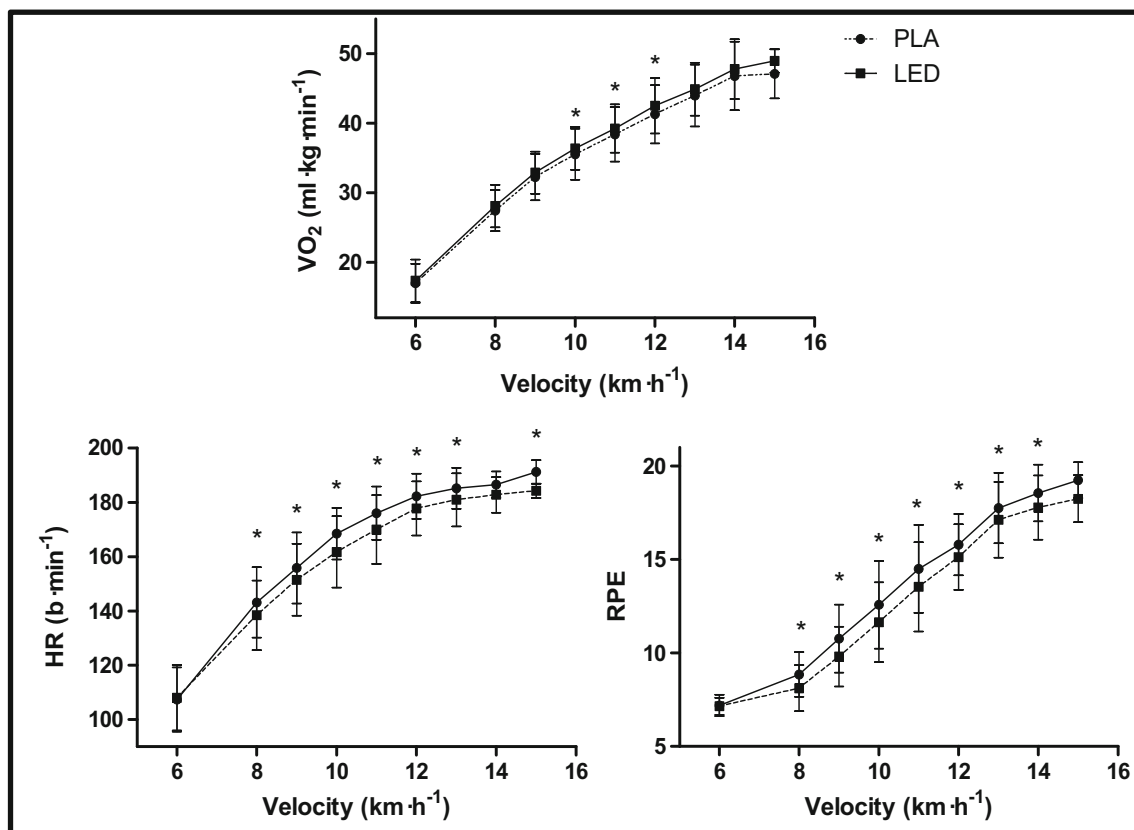


Fig. 2 Submaximal values of oxygen uptake (VO_2), heart rate (HR), and rating of perceived exertion (RPE) for placebo (PLA) and LED condition. $*p < 0.05$

physiological responses, which enhance time tolerance to fatigue during supra-threshold exercise intensities more than that observed for subthreshold intensities, because higher VO_{2sub} contributes to avoiding earlier metabolic perturbation. Studies that verified the effects of lights in a series of maximum muscle contractions corroborate this idea [9, 25].

The present study presented substantial alterations in HR_{max} comparing the LED and PLA condition, with an average reduction of two beats per minute at V_{peak} , in addition to a significant average reduction of HR_{sub} of up to seven beats per minute in intermediary incremental test stages, with statistically lower values for approximately 78% of the test total time in LED condition compared to PLA. Further studies also showed positive responses of light application for the HR recovery velocity and reduction of HR measured during incremental tests after a 6-month training period [7], and better cardiovascular efficiency caused by better peripheral extraction of O_2 [8].

The biological mechanisms of light can provide a better post-exercise recovery for cardiac parameters, possibly as better autonomic adjustments of HR and baroreflex activity [26]. Furthermore, studies demonstrated that the chronic response to the application of light could generate repetitive effects of peripheral vasodilation and increased induction of angiogenesis by release of nitric oxide and increased circulation by thermal effects [27, 28].

This study also demonstrated positive responses of the previous LED application in the blood lactate disappearance capacity; these effects could be due the improvement in microcirculation [6, 29] and also because of a decreased lactate production with prior application of light by modifications on oxidative capacity through enzymatic, mitochondrial, and systemic changes, facilitating the resynthesis of ATP by the alactic pathway [20, 21, 29]. Although there was no difference between conditions for LA_{peak} , LED condition presented higher removal capacity compared to PLA, with significantly lower values of LA_{13-min} and LA_{15-min} .

The moment of LA_{peak} achievement showed a contention capacity of LED to the increase in blood lactate concentrations after exercise compared to PLA, with 77% of subjects reaching LA_{peak} at the third post-test minute; in PLA condition, 42% of the subjects still had LA_{peak} concentrations reached between the fifth and ninth minutes. The application of light before the effort seems to be crucial to obtain positive effects on blood lactate disappearance [8, 18, 30] because apparently the effect of light on tolerance also improves blood lactate metabolism and the time to achieve peak response, which is expected to take more time as light sessions are applied after exercise [31].

Few studies investigated the effect of prior light sessions on RPE [7, 32], and no research used the Borg 6–20 scale. Using the 10 points scale, Felismino et al. [32] found no difference

for a placebo and prior laser session on the RPE during a muscle damage protocol, and Paolillo et al. [7] after 6 months of treadmill training found no differences for the placebo and LED groups on the reduction of RPE during the incremental step test. The pattern of prior LED session responses on HR, measured in this study, was linearly modified by the intensity and duration of the incremental step tests, similarly as RPE, suggesting that the Borg scale 6–20 is also responsive to changes in aerobic demand and therefore the effects of prior LED session.

Different prior exercise strategies can assist physical trainers and athletes to improve running performances and support training routine. The results revealed that LED sessions prior to running exercise modify cardiorespiratory response by affecting running tolerance during the incremental step test, as well as metabolite clearance. These responses also modified RPE during the entire test demonstrating psychophysiological effects of LED.

Despite the interesting results regarding LED application on HR and RPE, the present study also presented limitations that could have influenced the low effect size responses on VO_2 , time limit, and final velocity achieved during the incremental step test. Despite the selection of Caucasian subjects, skin color variation was not controlled, which can generate inter-subject variation. In addition, the LED equipment performance could vary with the usage time without an active feedback for power output control for each target muscle and subject.

However, in terms of practical application, LED prior to running exercise, as an acute strategy for healthy and physically active subjects, could be recommended to modulate oxidative response and enhance exercise tolerance, to reduce maximal and submaximal values of psychophysiological parameters, as the RPE and HR, and to accelerate blood lactate clearance, suggesting greater physical recovery after maximal exercises. Further research is also warranted to examine the chronic effects of LED application prior running training sessions.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

Ethical approval The study protocol was approved by the local ethics committee in research involving human beings—State University of Maringá (no. 147.362/2012).

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