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Pre-Exercise Infrared Low-Level Laser Therapy (810 nm) in Skeletal Muscle Performance and Postexercise Recovery in Humans, What Is the Optimal Dose? A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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Abstract

Aim: This study aimed to evaluate the medium-term effects of low-level laser therapy (LLLT or photobiomodulation) in postexercise skeletal muscle recovery and performance enhancement and to identify the optimal dose of 810 nm LLLT. ***Materials and methods:*** A randomized, double-blind, placebo-controlled trial was performed, with voluntary participation of 28 high-level soccer athletes. We analyzed maximum voluntary contraction (MVC), delayed onset muscle soreness (DOMS), creatine kinase (CK) activity, and interleukin-6 (IL-6) expression. The assessments were performed before exercise protocols, after 1 min, and 1, 24, 48, 72, and 96 h after the end of eccentric exercise protocol used to induce fatigue. LLLT was applied before eccentric exercise protocol with a cluster with five diodes, and dose of 10, 30, or 50 J (200 mW and 810 nm) in six sites of quadriceps. ***Results:*** LLLT increased ($p < 0.05$) MVC from immediately after exercise to 24 h with 50 J dose, and from 24 to 96 h with 10 J dose. Both 10 J then 50 J dose decreased ($p < 0.05$) CK and IL-6 with better results in favor of 50 J dose. However, LLLT had no effect in decreasing DOMS. No differences ($p > 0.05$) were found for 30 J dose in any of the outcomes measured. ***Conclusions:*** Pre-exercise LLLT, mainly with 50 J dose, significantly increases performance and improves biochemical markers related to skeletal muscle damage and inflammation.

Introduction

The skeletal muscles show a progressive decline of performance during strenuous physical activity/exercises, but the muscles recover fairly quickly after a period of rest. This reversible phenomenon is called muscle fatigue.[1] Muscle fatigue can be commonly divided into a central component and a peripheral component.

There are several different types of muscle fatigue, and the contribution of each type to the overall decline in muscle performance depends on the muscle fiber type, intensity, and duration of the activity.[2] However, it is not possible to confirm the etiology of the fatigue by classic tests such as maximal voluntary contraction (MVC), since this requires appropriated experimental conditions to verify the influence of the neural and peripheral components.[3] Enoka and Duchateau[3] defined fatigue as ''a disabling symptom in which physical and cognitive function is limited by interactions between performance fatigability and perceived fatigability,'' and therefore, it is hard to suggest where the main changes are occurring.[3]

Muscle damage can occur in sports or in other activities as a result of skeletal muscle fatigue development.[4] The evaluation of muscle damage in humans is difficult and

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complex. Direct analysis is possible only through muscle biopsy or magnetic resonance imaging; however, both methods are expensive and have questionable diagnostic accuracy.[5] The monitoring of serum activity of skeletal muscle enzymes is currently widely used to assess muscle damage.[6] The most common changes in protein and enzyme activity after exercises are creatine kinase (CK), lactate dehydrogenase, aspartate transaminase, and myoglobin.[7] However, the plasmatic activity of CK appears to be the best indicator of exercise intensity and the effects on muscle tissue.[8]

High-intensity and repetitive skeletal muscle contractions can also induce a protective inflammatory response, which is normally related to skeletal muscle damage.[9–11] The initiation of primary muscle damage induced by exercise may be fatiguing but is not painful. However, the ensuing inflammatory response leads to delayed onset muscle soreness (DOMS) beginning 8–24 h after the damage is initiated.[12,13] Primary muscle tissue damage promotes infiltration by inflammatory cells, which in conjunction with local muscle, endothelial, and satellite cells, produce an array of cytokines to regulate the inflammatory process, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6.[13–15]

Currently, there are many therapeutic modalities used after sports activities to improve skeletal muscle recovery. The most commonly modalities used are as follows: active recovery,[4,16–18] cryotherapy,[4,19,20] massage,[17,21] contrast heat therapy (use of hot and cold water immersion),[22,23] hydrotherapy,[24] stretching,[25] and electrostimulation.[26] However, the scientific evidence behind these modalities is limited.

It has been hypothesized that low-level laser therapy (LLLT) promotes tissue regeneration, reduces inflammation, and relieves pain.[27] Skeletal muscle fatigue and recovery is a novel area of research in LLLT. Recent studies of our research group with LLLT and light emitting diode therapy have shown positive results delaying skeletal muscle fatigue in both animals and humans and improving the status of biochemical markers related to skeletal muscle recovery when these therapies were applied before exercise.[28–35] Despite positive effects observed, several factors still remain unknown, such as mechanisms, optimal dose, effects in long-duration exercises, and long-term effects in skeletal muscle recovery.[35]

It is known that LLLT has a biphasic dose–response pattern, which follows the Arndt–Schulz Law. Biostimulatory effects can be achieved using doses within a dose range, also known as therapeutic window. Inhibitory effects are observed when doses above this therapeutic window are used, and in the same way, no effects are observed when doses below the therapeutic window are used. Therefore, the establishment of optimal doses and therapeutic windows for different pathologies and conditions becomes crucial for optimization of LLLT. With this perspective in mind, the aim of this study was to identify the optimal dose for preexercise irradiation with LLLT looking for performance enhancement and improvement of postexercise recovery, through functional and biochemical markers related to muscle damage.

Materials and Methods

Study design and ethics statement

A double-blind, placebo-controlled, randomized clinical trial was carried out in two phases. The study was conducted in the Laboratory of Phototherapy in Sports and Exercise at Universidade Nove de Julho in the city of São Paulo, Brazil. The project has received approval from the Research Ethics Committee of University Nove de Julho (Protocol No. 397774/2011). The protocol for this study is registered with the Protocol Registry System (clinicaltrials.gov; NCT01844271).

Characterization of sample

Twenty-eight male professional soccer athletes from the same team participated in the study. They had an average age of 18.81 ± 0.80 years old, height of 172.94 ± 4.48 cm, and body weight of 63.58 ± 4.46 kg. The decision to recruit volunteers from the same team was made to enhance the homogeneity of the sample. Moreover, the tests for this study were performed with the athletes during preseason preparation. Therefore, the whole sample performed all procedures at the same physical activity level.

Calculation of sample size

The sample size was calculated based on a previous study carried out in same research field,[36] in which a similar experimental model and exercise protocol were employed. The sample size calculation considered a β of 20% and α of 5%. We used as reference for this calculation the study performed by Baroni et al.,[36] where LLLT led to the postexercise recovery of CK (muscle injury marker) to 435.95 ± 238.04 U/L, whereas placebo treatment led to an increase in CK to 1327.58 ± 949.82 U/L. Using these parameters, a total of seven volunteers were needed for each of the four groups in study (total of 28 volunteers). The intention-to-treat analysis was followed *a priori*. CONSORT flowchart summarizing experimental procedures and subjects is shown in Fig. 1.

Inclusion criteria

The following inclusion criteria were used:

- Professional football athletes;
- Age between 18 and 35 years;
- Male gender;
- Minimum of 80% participation in team practice sessions;
- Light or intermediate skin color, following Von Luschan's chromatic scale[37];
- Agreement to participate through signed statement of informed consent.

Exclusion criteria

Participants with the following were excluded from the study:

- History of musculoskeletal injury to hips or knees in the previous 2 months;
- Use of pharmacological agents or nutritional supplements;
- Smokers and alcoholics;
- Occurrence of musculoskeletal injury during the study;
- Any change in practice routine in relation to the rest of the team during the study.

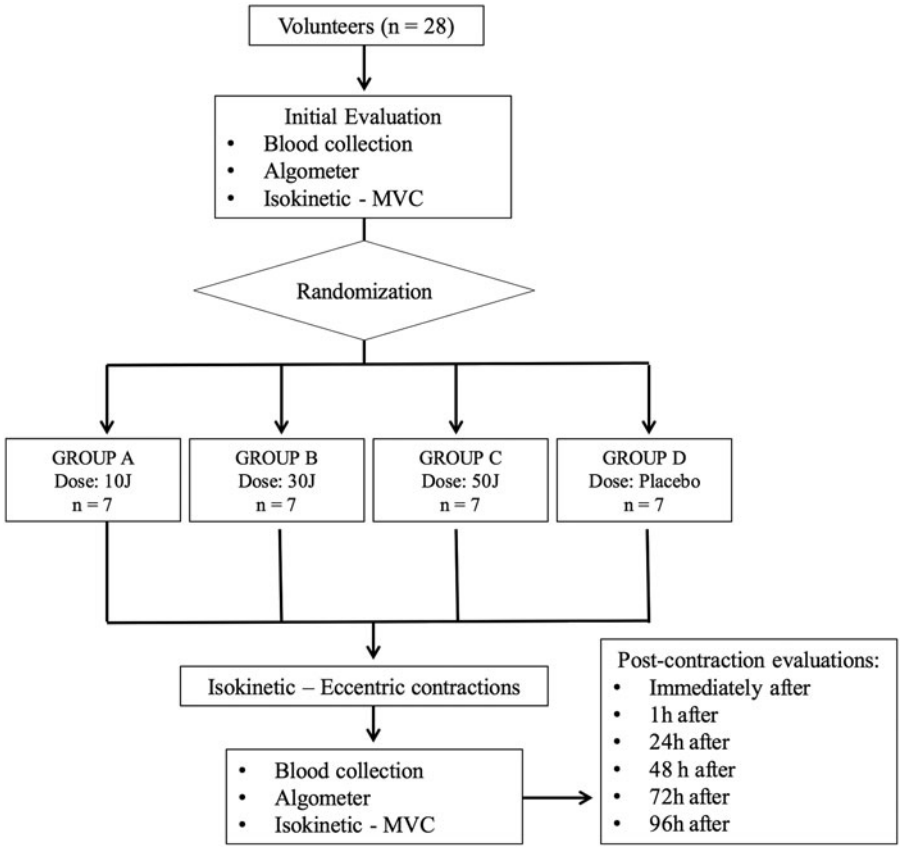

FIG. 1. CONSORT flowchart.

Composition of groups and randomization process

The volunteers were randomly allocated to four experimental groups ($n = 7$ per group) according to the LLLT dose. Randomization was carried out by a simple drawing of lots (A, B, C, or D). The laser unit emitted the same sound regardless of the programmed dose. Randomization labels were created using a randomization table at a central office, where a series of sealed, opaque, and numbered envelopes were used to ensure confidentiality. A participating researcher who programmed the laser device based on the randomization results conducted randomization. This researcher was instructed not to inform the participants or other researchers regarding the LLLT dose.

The researcher in charge of the administration of the LLLT was blinded to the dose applied to the volunteers, and therefore, one of the researchers involved in study programmed laser device unit according to randomization while another one performed the administration of the light therapy. Researchers who did not have knowledge about randomization performed the assessments and exercise protocol. Blinding was further maintained by the use of opaque goggles by the participants.

Experimental protocol

Evaluations and informative procedures. Evaluations were carried out before and at the end of the isokinetic protocol by a researcher blinded to the LLLT dose and mode (placebo or active). The volunteers were then informed about the procedures and signed a statement of informed consent in compliance with Resolution 196/96 of the Brazilian National Board of Health before the execution of the study.

Blood samples and biochemical analyses. Following the informative process and randomization, blood samples (10 mL) were taken from the antecubital vein before and 1 min after the eccentric contraction protocol. Blood samples were also collected 1, 24, 48, 72, and 96 h after the protocol. The samples were taken by a qualified nurse blinded to the allocation of the volunteers to the four experimental groups. One hour after collection, each sample was centrifuged at 3000 rpm for 20 min. Pipettes were used to transfer the serum to Eppendorf® tubes, which were stored at −80°C until analysis.

Blood analysis involved the determination of CK activity as an indirect marker of muscle damage using spectrophotometry and specific reagent kits (Labtest®, São Paulo, Brazil); and IL-6 levels as inflammatory marker using ELISA method and specific reagents (BD, San Diego, CA). The researcher who performed analysis of biochemical markers was blinded to randomization and allocation of volunteers in experimental groups.

Evaluation of DOMS. DOMS was evaluated based on the pressure pain threshold using an analog algometer (Baseline®, Parma, Italy). This device consists of a rod with a rounded rubber tip coupled to a pressure (force) meter. The display presents values in pounds (lbs). As the surface of the rubber tip measures 1 cm^2, the reading is expressed in pounds per square centimeter (lbs/cm^2). Values range from

0 to 100 lbs with a precision of 0.1 lbs. The most sensitive areas of the knee extensors (medial, lateral, and central) of the nondominant lower limb were located through palpation by an examiner blinded to the allocation of the volunteers to the different groups and were marked with a dermographic marker. The cylindrical end of the equipment was positioned perpendicularly to the demarcated area. Pressure was applied to the surface of the skin with a gradual increase in increments of 0.1 lbs.

The volunteers were instructed to say ''yes'' when the pressure exerted becomes painful. Three measures were taken with the algometer on the same demarcated point of the aforementioned muscle sites. The mean pressure pain threshold was determined from the three readings of each of the three sites and the mean values were used in the statistical analysis. Readings were taken before stretching and warm up, 1 min after the eccentric contraction protocol as well as 1, 24, 48, 72, and 96 h after the execution of the protocol.

To evaluate DOMS, we also used a visual analog scale (VAS) of 100 mm. VAS consisted in a 100 mm empty line with the word ''no pain'' on the left side (at the beginning of the line) and ''worst pain imaginable'' on the right side (at the end of the line). The line was always presented horizontally to the volunteers and they were asked to indicate the pain intensity in the line. After that, a researcher measured the distance between the beginning of the line until the volunteer indication, to quantify the pain intensity. The researcher who performed assessment of DOMS was blinded to randomization and allocation of volunteers in experimental groups.

Stretching and warm up. Before the isokinetic protocol, the volunteers performed three 60-sec sets of active stretching of the knee extensors of the nondominant lower limb. The volunteers then performed a warm-up exercise consisting of pedaling a stationary bike (Ibramed®, Porto Alegre, Brazil) at 100 rpm for 5 min without load.

Isometric protocol test: maximum voluntary contraction. An isokinetic dynamometer was used for the evaluation of muscle function and the execution of the exercise protocol. This instrument is currently considered the method with the greatest reliability for the measure of the musculoskeletal performance.

Immediately after the stretching and warm-up exercises, the MVC test was performed. For such, the volunteers sit on the seat of the isokinetic dynamometer (System 4; Biodex®, Shirley, NY) with an angle of 100° between the trunk and hip and the nondominant leg positioned with the knee at 60° of flexion (0° corresponds to complete knee extension) and attached to the seat of the dynamometer by straps. The dominant leg was positioned at 100° of hip flexion and was also attached to the seat by a strap. The volunteers were also attached to the seat of the dynamometer through the use of two straps crossing the trunk.

The volunteers were instructed to cross their arms over the trunk and the axis of the dynamometer was positioned parallel to the center of the knee. The MVC test consisted of three 5-sec isometric contractions of the knee extensors of the nondominant leg. The highest torque value of the three contractions (peak torque) was used for the statistical analysis. The choice of this parameter is due to the fact that this variable reflects the maximum generation of force by the muscle. Instructions on how to execute the test were given first and the volunteers received verbal encouragement during the execution of the test.

This test has demonstrated reliability and reproducibility in a previous studies carried out by our research group.[36,38] The MVC was performed also immediately (1 min) after the eccentric contraction protocol as well as 1, 24, 48, 72, and 96 h after the eccentric contraction protocol to evaluate postexercise muscle recovery. The researcher who performed assessment of MVC was blinded to randomization and allocation of volunteers in experimental groups.

Low-level laser therapy. A five-diode cluster laser device (manufactured by Thor Photomedicine®, London, United Kingdom) was used for LLLT. To ensure blinding, the device emitted the same sounds regardless of the programmed mode (active or placebo). The optical power was calibrated before irradiation in each volunteer using a Thorlabs thermal power meter (Model S322C; Thorlabs®, Newton, NJ).

LLLT was applied 2 min before the pre-exercise MVC test with the cluster in direct contact with the skin at six distinct sites of the knee extensor musculature of the nondominant limb (two medial, two lateral, and two central sites; Fig. 2). As the cluster has 5 diodes and 6 different sites were irradiated, a total of 30 points were irradiated in the musculature. The use of a cluster in this irradiation procedure is important since this allowed us to cover larger areas of irradiation. Based on the results of the randomization, the volunteers of the four experimental groups received the following doses:

FIG. 2. Sites of low-level laser therapy irradiation on quadriceps.

- Group A—60 J of total irradiated energy on knee extensors (six sites in the muscle with a five-diode probe and 2 J/diode, 10 J in each site) with 10 sec of irradiation at each site (60 sec of total irradiation time);
- Group B—180 J of total irradiated energy on knee extensors (six sites in the muscle with a five-diode probe and 6 J/diode, 30 J in each site) with 30 sec of irradiation at each site (180 sec of total irradiation time);
- Group C—300 J of total irradiated energy on knee extensors (six sites in the muscle with a five-diode probe and 10 J/diode, 50 J in each site) with 50 sec of irradiation at each site (300 sec of total irradiation time);
- Group D—0 J of total irradiated energy on knee extensors (six sites in the muscle with a five-diode probe and 0 J/diode, 0 J in each site), with 20 sec of irradiation at each site, 120 sec of total time, but without effective irradiation.

The researcher who irradiated LLLT was blinded to randomization and allocation of volunteers in experimental groups. The irradiation sites are illustrated in Fig. 2 and the parameters for LLLT are shown in Table 1.

Isokinetic protocol: eccentric contractions. Precisely 3 min after the end of LLLT, the volunteers performed the eccentric contraction protocol, which consisted of 75 eccentric isokinetic contractions of the knee extensor musculature in the nondominant leg (5 sets of 15 repetitions, 30-sec rest interval between sets) at a velocity of $60°.seg^{-1}$ in both the eccentric and concentric movements with a 60° range of motion (between 90° and 30° of knee flexion). At each contraction, the dynamometer automatically (passively) positioned the knee at 30°; the dynamometer then flexed the knee until reaching 90°.

The volunteers were instructed to resist against knee flexion movement imposed by the dynamometer with maximum force. Instructions on how to execute the maneuver were given first and the volunteers received verbal encouragement throughout the protocol. Volunteers performed five submaximal repetitions as familiarization procedure before tests. The dominant leg was determined by asking volunteers about the preferred leg to kick a ball, and then, tests were performed with the nonpreferred leg (nondominant). Despite the diversity of protocols proposed for the execution of eccentric exercises on isokinetic dynamometers, the protocol described here was chosen based on two previous studies carried out in the same research field,[36,38] in which this protocol proved effective and reproducible for the exercise-induced muscle damage.

The researcher in charge to eccentric contractions protocol was blinded to randomization and allocation of volunteers in experimental groups.

Table 1. Low-Level Laser Therapy Parameters

Wavelength, nm	810 (infrared)
Number of diodes	5
Power output per diode, mW	200 (total of 1000)
Power density per diode, W/cm^2	5.495
Energy per diode, J	2, 6, or 10
Energy per site, J	10, 30, or 50
Energy density per diode, J/cm^2	54.95, 164.84, 274.73
Spot size, cm^2—each diode	0.0364
Treatment time per point or site, sec	10, 30, or 50
Total treatment time, sec	60, 180, or 300
Total energy delivered, J	60, 180, or 300
Number of treated points/sites	30 points/6 sites
Application mode	Probe held stationary in skin contact at a 90° angle with slight pressure

Statistical analysis

Data were first tested regarding normal distribution using the Shapiro–Wilk test and are expressed as mean and standard deviation since it has normal distribution. The ANOVA test with repeated measurements for the time factor was performed to test between-group differences (followed by Bonferroni *post hoc* test). The significance level was set at $p < 0.05$. Data in graphs are expressed as mean and standard error of the mean. The researcher who performed statistical analysis was blinded to randomization and allocation of volunteers in experimental groups. *A priori,* an intention to treat basis would be followed, however, it was not performed since there were not dropouts.

Results

All athletes recruited completed all assessments performed in the study, and therefore, there were no dropouts. Table 2 shows all outcomes regarding functional aspects of performance and recovery that we observed in our study. As we can observe, there were no significant differences ($p < 0.05$) between experimental groups regarding DOMS both in algometry and in VAS.

On the contrary, 10 J LLLT dose significantly increased ($p < 0.05$) MVC compared to placebo both in absolute and in percentage values at 24, 48, 72, and 96 h. In addition, 50 J LLLT dose significantly increased ($p < 0.05$) MVC compared to placebo both in absolute and in percentage values immediately after eccentric exercise protocol and at 1 and 24 h. Figures 3 and 4 show results regarding MVC in absolute and in percentage values.

Our CK analysis shows that 10 J LLLT dose significantly decreased ($p < 0.05$) CK activity compared to both placebo and 30 J LLLT dose at 24, 48, 72, and 96 h after eccentric contractions protocol. Interestingly, 50 J LLLT dose significantly decreased ($p < 0.05$) CK activity compared to both placebo and 30 J LLLT dose at 1, 24, 48, 72, and 96 h after eccentric contractions protocol. In contrast, 30 J LLLT dose did not show significant difference ($p > 0.05$) compared to placebo LLLT in all time points tested. Results regarding CK analysis are summarized in Fig. 5.

Regarding inflammation, 30 J LLLT dose significantly decreased ($p < 0.05$) IL-6 levels compared to placebo immediately after eccentric exercise protocol and at 1, 24, 48, and 72 h. Similarly, 50 J LLLT dose significantly decreased ($p < 0.05$) IL-6 levels compared to placebo at 1, 24, 48, and 72 h. However, only 10 J LLLT dose significantly decreased ($p < 0.05$) IL-6 levels compared to placebo at all time points tested (immediately after eccentric exercise protocol, and at 1, 24, 48, 72, and 96 h). Figure 6 summarizes results regarding IL-6 levels.

TABLE 2. FUNCTIONAL MARKERS OF PERFORMANCE AND RECOVERY (MEAN ± SD)

	Pre	*Post*	*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
VAS, mm							
Placebo	0.00 ± 0.00	68.30 ± 17.20	21.39 ± 20.31	45.00 ± 30.20	25.00 ± 13.80	18.30 ± 11.70	29.78 ± 30.75
10 J	0.00 ± 0.00	43.33 ± 15.05	32.17 ± 19.92	41.66 ± 32.50	26.66 ± 30.11	28.33 ± 30.60	50.78 ± 29.79
30 J	0.00 ± 0.00	48.00 ± 8.40	58.00 ± 14.80	46.00 ± 20.70	46.00 ± 24.10	30.00 ± 21.20	28.00 ± 27.70
50 J	0.00 ± 0.00	48.00 ± 13.03	52.00 ± 19.23	44.00 ± 24.08	48.00 ± 33.46	28.00 ± 19.23	22.50 ± 22.17
Algometry, lbs							
Placebo	30.00 ± 5.52	28.53 ± 7.66	27.06 ± 10.64	30.93 ± 10.46	31.26 ± 12.86	33.40 ± 11.98	34.80 ± 14.19
10 J	27.85 ± 29.78	25.65 ± 10.76	24.38 ± 5.73	26.09 ± 6.85	29.62 ± 11.34	27.90 ± 8.13	28.14 ± 7.01
30 J	28.67 ± 5.28	26.07 ± 6.28	20.00 ± 1.20	20.87 ± 5.66	25.53 ± 4.05	31.13 ± 6.88	33.00 ± 7.68
50 J	24.13 ± 12.21	25.06 ± 11.02	25.13 ± 11.17	24.31 ± 10.43	26.60 ± 8.98	29.60 ± 12.44	26.25 ± 10.13
MVC, N.m							
Placebo	249.90 ± 22.65	228.14 ± 13.57	213.86 ± 29.00	247.40 ± 11.40	249.72 ± 28.28	243.86 ± 12.41	256.86 ± 8.52
10 J	253.32 ± 24.53	226.67 ± 15.35	238.41 ± 10.00	286.77 ± 22.78[ab]	294.31 ± 21.75[abc]	292.08 ± 20.71[ab]	305.57 ± 23.30[ab]
30 J	246.79 ± 23.61	220.83 ± 24.00	215.91 ± 6.36	223.44 ± 9.23	242.11 ± 7.90	228.44 ± 12.73	240.79 ± 18.72
50 J	249.78 ± 15.71	259.04 ± 19.43[abd]	262.17 ± 20.08[ab]	275.97 ± 12.21[ab]	261.92 ± 27.32	270.07 ± 13.43[b]	281.22 ± 22.14[b]

[a]Different of placebo ($p < 0.05$).
[b]Different of 30 J ($p < 0.05$).
[c]Different of 50 J ($p < 0.05$).
[d]Different of 10 J ($p < 0.05$).
MVC, maximum voluntary contraction; VAS, visual analog scale.

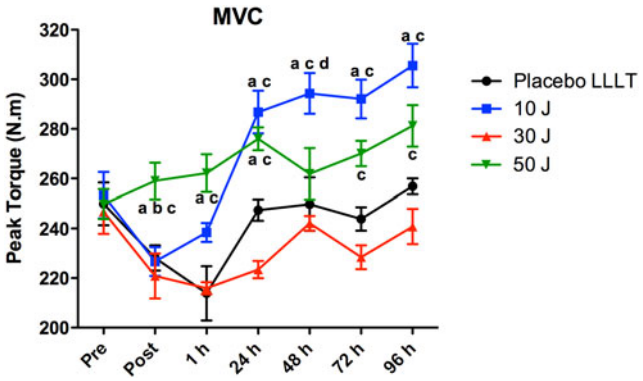

FIG. 3. MVC in absolute values. Values are mean and error bars are SEM. [a]Different of placebo ($p < 0.05$); [b]Different of 10 J ($p < 0.05$); [c]Different of 30 J ($p < 0.05$); [d]Different of 50 J ($p < 0.05$). MVC, maximum voluntary contraction; SEM, standard error of the mean.

Discussion

As far as we know, this is the first time that several LLLT doses with infrared 810 nm wavelength are tested in same experiment to evaluate effects on exercise performance and postexercise recovery in high-level athletes. We decided to evaluate three different doses to help establish a ''therapeutic window'' for LLLT in performance and recovery enhancement.

We choose to irradiate muscles before exercise, since several studies have shown that when pre-exercise LLLT is used, it has ergogenic effects and protects muscles against damage. Recently, a systematic review with meta-analysis has stated the same in its conclusions.[35]

Interestingly, two doses tested (10 and 50 J) showed significant results in improvement of MVC, but at different times. Dose 10 J resulted in a significant increase in muscle strength compared to the placebo group from 24 to 96 h after eccentric contractions protocol, and dose of 50 J resulted in a significant increase in muscle strength compared to placebo group from immediately postexercise to 24 h after eccentric contractions protocol. However, 30 J dose does not show any significant effect. We believe that different doses

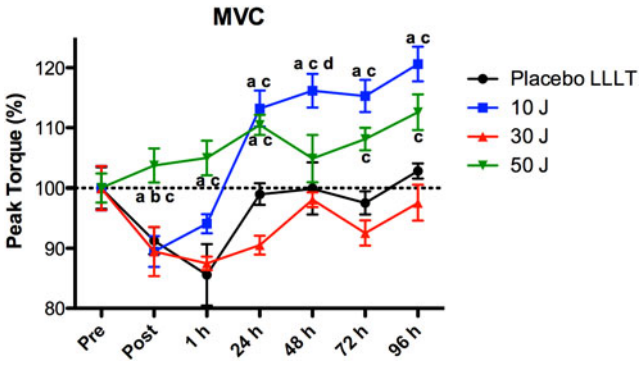

FIG. 4. MVC in percentage values. Values are mean and error bars are SEM. [a]Different of placebo ($p < 0.05$); [b]Different of 10 J ($p < 0.05$); [c]Different of 30 J ($p < 0.05$); [d]Different of 50 J ($p < 0.05$).

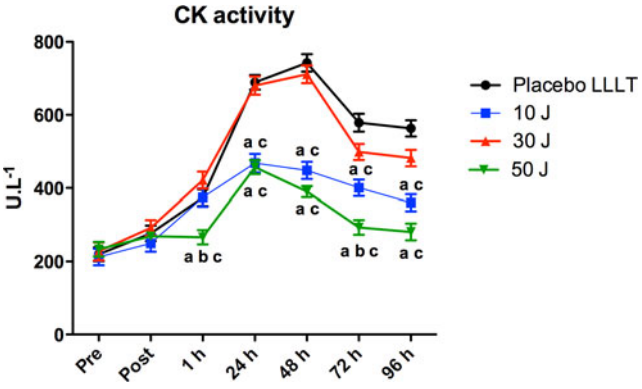

FIG. 5. CK activity. Values are mean and error bars are SEM. [a]Different of placebo ($p < 0.05$); [b]Different of 10 J ($p < 0.05$); [c]Different of 30 J ($p < 0.05$); [d]Different of 50 J ($p < 0.05$). CK, creatine kinase.

used in our study can lead to different time-windows (which can explain the immediate and delayed responses promoted by different doses) and/or different mechanisms of action. However, these points warrant further investigation.

These results could be very helpful thinking about sports modalities, for instance, in sports, such as swimming, judo, and short-distance running, an immediate or short-term recovery is required, and therefore, 50 J dose would be the best dose to be used in athletes. On the contrary, in sports, such as football, basketball, and volleyball, the medium-term recovery (from 48 to 96 h) is needed, and therefore, the best dose to be used would be 10 J. Curiously, our results regarding MVC are very different than those observed by Baroni et al.[36] Authors used an 810 nm LLLT, 200 mW, and only tested a single dose of 30 J with the same other parameters (power density, energy density and irradiation time) used in the current study; however, they observed a significant improvement in muscle performance immediately after and at 24 and 48 h after exercise.

Antonialli et al.[38] also tested the same three doses we tested (10, 30, and 50 J) but using a different device that simultaneously uses different light sources and wavelengths (super-pulsed laser of 905 nm, red LED of 640 nm, and infrared LED of 875). Untrained volunteers were recruited and

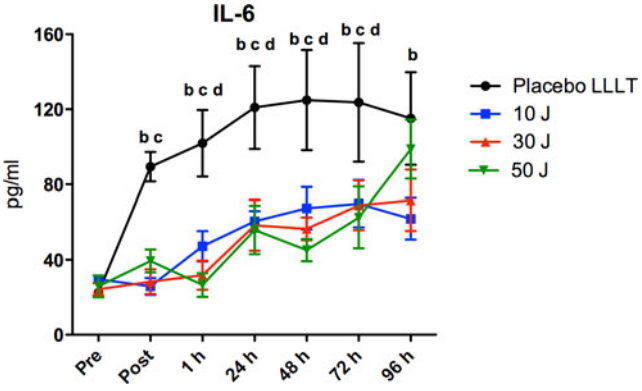

FIG. 6. IL-6 levels. Values are mean and error bars are SEM. [a]Different of placebo ($p < 0.05$); [b]Different of 10 J ($p < 0.05$); [c]Different of 30 J ($p < 0.05$); [d]Different of 50 J ($p < 0.05$). IL-6, interleukin-6.

it was found that there were better results regarding MVC enhancement, decrease in DOMS, and decrease in CK activity with 30 J dose applied per site before exercise.

This difference could be related to some aspects such as the sample selected, high-level male football athletes versus healthy male volunteers (nonathletes),[36,38] or the device used, single wavelength versus multiple wavelengths.[38] However, further studies are also needed to investigate these aspects.

Our results also show that 10 and 50 J doses significantly decreased CK activity, with best results in favor of 50 J. However, 30 J dose again showed no effect compared to placebo. LLLT also decreased the serum CK in the study performed by Dos Reis et al.[39] The effect was more pronounced when LLLT was applied after the fatigue protocol used by authors. They recruited professional football players, but the device and parameters used were very different from those chosen in our study. Furthermore, the irradiation time used by Dos Reis et al.[39] was very limited (10 sec) to achieve significant effects for LLLT before and/or after exercise.

Also, our results are very different than those observed by Baroni et al.,[36] and we believe that this difference may be due subjects' characteristics (high-level athletes vs. nonathletes). It is known that athletes normally present different plasma CK activities than nontrained individuals[40] and that postexercise CK activity also increases differently in athletes compared to nontrained subjects,[41] which could explain this difference in results regarding CK compared with the results of Baroni et al.[36]

Interestingly, all doses tested significantly decreased IL-6 expression. This result is in line with previous studies performed by our research group,[31,34] where we observed that pre-exercise phototherapy significantly decreased c-reactive protein levels. Over the years, several animal and human trials have shown that LLLT with both red and infrared wavelengths has modulatory effects on inflammatory marker release (PGE_2, TNF-α, IL-1β, plasminogen activator)[42] and several phases of the inflammatory process itself (edema, hemorrhagic formation, necrosis, neutrophil cell influx) and leukocyte activity (macrophages, lymphocytes, neutrophils).[43–47] This includes inhibition of the NF-kappa pathway[48] and modulation of inducible nitric oxide synthase.[49]

It is important to highlight that assessments performed in this study do not allow us to explore mechanisms of action nor if effects on performance enhancement are related to delayed central or peripheral fatigue. Therefore, to avoid overstatements or speculation on observed outcomes, we believe that these aspects should be investigated in further studies in this field.

Hayworth et al.[50] demonstrated that a single irradiation with LLLT is able to increase the cytochrome c-oxidase activity in intact skeletal muscle tissue 24 h after irradiation. In addition, authors demonstrated that there is a dose- and fiber-type-dependent increase in cytochrome c-oxidase in skeletal muscle fibers. It means that LLLT leads to upregulation of mitochondrial activity through increasing mitochondrial respiratory chain, which consequently increases ATP production into muscle cells and decreases oxidative stress and reactive oxygen species production. These effects can explain the mechanism through LLLT enhances performance and protects skeletal muscle against damage and inflammation.

Very recently, Albuquerque-Pontes et al.[51] investigated the effects of different doses (1, 3, and 10 J) and wavelengths (660, 830, and 905 nm) in cytochrome c oxidase activity in intact skeletal muscle. They concluded that parameters which increased the cytochrome-c oxidase were mainly 660 nm at 1 J, 830 nm at 3 J, and 905 nm at 1 J. The increase in cytochrome-c oxidase was observed from 5 min up to 24 h after irradiation, depending upon irradiation parameters used. This demonstrates that phototherapy can be used in different time-windows between irradiation and beginning of muscular activity, and it is dependent of wavelengths and doses used.

This agrees with the previous observation by Santos et al.,[52] who observed that ergogenic and protective effects of LLLT on skeletal muscle are also dependent of wavelengths and doses used. These results help us to elucidate how pre-exercise phototherapy improves performance,[35,38,53,54] delays fatigue development, and can protect muscle against damage even in difficult diseases such as muscular dystrophies.[55,56]

Despite positive results observed in muscle strength and biochemical markers of muscle damage and inflammation, none of LLLT doses tested showed significant results in decreasing DOMS. Interestingly, the same was observed in a previous study using the same device and single wavelength.[36] In contrast, the combination of multiple wavelengths showed positive results in decreasing DOMS.[38] Therefore, the effect of LLLT with single wavelength on DOMS is still an open issue and deserves further investigation.

Conclusions

Pre-exercise LLLT significantly increases performance and improves biochemical markers related to skeletal muscle damage and inflammation. Better results were observed with 10 and 50 J doses. The overall analysis of results shows that better results are reached with 50 J dose.

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