

Effects of Pre- or Post-Exercise Low-Level Laser Therapy (830 nm) on Skeletal Muscle Fatigue and Biochemical Markers of Recovery in Humans: Double-Blind Placebo-Controlled Trial

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Abstract

Objectives: The purpose of this study was to investigate the effect of low-level laser therapy (LLLT) before and after exercise on quadriceps muscle performance, and to evaluate the changes in serum lactate and creatine kinase (CK) levels. **Methods:** The study was randomized, double blind, and placebo controlled. **Patients:** A sample of 27 healthy volunteers (male soccer players) were divided into three groups: placebo, pre-fatigue laser, and post-fatigue laser. The experiment was performed in two sessions, with a 1 week interval between them. Subjects performed two sessions of stretching followed by blood collection (measurement of lactate and CK) at baseline and after fatigue of the quadriceps by leg extension. LLLT was applied to the femoral quadriceps muscle using an infrared laser device (830 nm), 0.0028 cm² beam area, six 60 mW diodes, energy of 0.6 J per diode (total energy to each limb 25.2 J (50.4 J total), energy density 214.28 J/cm², 21.42 W/cm² power density, 70 sec per leg. We measured the time to fatigue and number and maximum load (RM) of repetitions tolerated. Number of repetitions and time until fatigue were primary outcomes, secondary outcomes included serum lactate levels (measured before and 5, 10, and 15 min after exercise), and CK levels (measured before and 5 min after exercise). **Results:** The number of repetitions ($p=0.8965$), RM ($p=0.9915$), and duration of fatigue ($p=0.8424$) were similar among the groups. Post-fatigue laser treatment significantly decreased the serum lactate concentration relative to placebo treatment ($p<0.01$) and also within the group over time (after 5 min vs. after 10 and 15 min, $p<0.05$ both). The CK level was lower in the post-fatigue laser group ($p<0.01$). **Conclusions:** Laser application either before or after fatigue reduced the post-fatigue concentrations of serum lactate and CK. The results were more pronounced in the post-fatigue laser group.

Introduction

SKELETAL MUSCLE FATIGUE (SMF) IS an inevitable phenomenon in routine athletic training and competitions that can degrade performance and predispose the athlete to a variety of musculoskeletal disorders.¹ Although SMF is also very common in activities of daily life, the underlying mechanisms of its action and development and the best preventive measures remain unclear.² This type of damage may be

transient, lasting only minutes or hours after exercise, but can also persist for several days. Some common features of SMF are decreases in muscle strength and motor control as well as muscle pain.³

Power generation decreases under the anaerobic conditions that inevitably occur during extensive exercise. Such conditions produce large amounts of reactive oxygen species (ROS), which are known to cause mitochondrial depolarization and affect mitochondrial function and also to reduce

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strength.³ Despite this, high-level monitoring of the concentration of lactic acid in the blood is still the main tool used to plan training programs in most sports.⁴

Serum levels of muscle enzymes are markers of the functional state of muscle tissue and vary greatly under both physiological and pathological conditions. The initial increases in the levels of these enzymes are associated with cell necrosis and tissue damage after acute and chronic muscle injury.⁵

A large number of therapeutic modalities are used in sports rehabilitation to accelerate muscle recovery after exercise; these include active recovery,⁶ cryotherapy,⁷ massage therapy,⁸ contrast baths (immersion in hot and cold water),⁹ hydrotherapy,¹⁰ stretching,³ hyperbaric oxygen therapy,¹¹ nonsteroidal anti-inflammatory drugs (NSAIDs),¹² and electrostimulation.¹³

Treatment of SMF is a new area of research for low-level laser therapy (LLLT), and the ideal parameters for the application are not yet fully understood.

Studies in humans show a trend toward improvement in muscle performance in response to laser therapy; however, some researchers¹⁴ have reported that applying the laser before the fatigue-inducing exercise provides more satisfactory reduction of fatigue, whereas others¹⁵ have obtained meaningful improvements in performance with laser application after the induction of fatigue. Therefore, the optimal moment to perform irradiation (before, during, or after exercise) still is an open and unanswered issue. On the other hand, recently a systematic review was published, and its authors stated that phototherapy with lasers and light-emitting diodes (LEDs) had ergogenic effects and protected muscles when applied before muscle activity.¹⁶

Decreasing muscle fatigue is now known to be an important way for high-performance and even amateur/recreational athletes to increase their performance levels naturally, and is also valuable for persons with pathological conditions that increase fatigue.

Therefore, this study was performed to confirm that LLLT is a useful new treatment modality, elucidate the ideal conditions, and, especially, determine whether LLLT is best applied as a preventive measure or as a treatment for existing muscle fatigue (i.e., pre- or post-exercise). The effects of LLLT performed before and after fatigue on quadriceps muscle performance were examined by implementing a fatigue protocol to induce muscle fatigue of the lower limbs (LL) in human subjects, in two intervention sessions (1 week interval between), and the serum levels of CK and lactate were evaluated before and after the application of LLLT.

Methods

Participants

The study was randomized, double blind, and placebo controlled, and was approved by the Ethics Committee of UFMS. Before the start of the experiment, all volunteers were informed about the research procedures and signed the Declaration of Informed Consent in accordance with the Guidelines and Standards for Research Involving Human Subjects in the Resolution of the National Board of Health no. 196/96.

All volunteers were recruited from the professional soccer team Centro Esportivo Nova Esperança (CENE-MS) of

Campo Grande, MS, Brazil. We used the following inclusion criteria: male sex, age from 15 to 30 years, playing soccer at the professional level, at least 4 years' experience practicing football, and training at least 5 days per week. The exclusion criteria were any prior musculoskeletal injury to the knee, hip, or ankle; participation in <80% of the football team's regular physical training, the use of any nutritional supplement or pharmacological agent, and the presence of any contraindication to the use of LLLT.

The data were collected at the Center Specializing in Sports Physiotherapy (CEFE) located in Clinica Orthos, in February 2013.

Randomization procedure

Randomization was performed by a simple drawing of lots (A or B), which determined whether participants would receive the active LLLT or placebo treatment in the first session. The randomization was performed by an assistant who was not involved with the experiment. The code allocation key was given to a technician, who set the control unit of the laser to the active or placebo setting.

After this step, the technician handed the preset laser to the therapist. The technician was instructed not to communicate the type of treatment assigned to either the subject or the therapist. Therefore, the treatment allocation was hidden from the subjects, therapists, and observers.

Experimental groups

We used a prototype 830 nm Gallium-Aluminum Arsenide (GaAlAs) laser device, manufactured by DMC[®], Sao Carlos – Brazil, which had six 60 mW diodes, each with a beam area of 0.0028 cm², arranged in a single row array and operated in continuous mode. This linear laser array was applied seven times, thus irradiating 42 points, to each leg, for a total of 84 irradiated points per treatment session. Irradiation was performed with the laser in direct contact with the skin, and the laser array was kept stationary under slight pressure at an angle of 90 degrees to the surface of the skin. The belly of the quadriceps muscle was irradiated. We initially defined an area 10 cm below the anterior superior iliac spine as the location of the first set of application points, and the other six locations were set to standardized points 5 cm below the initial marks (Fig. 2). The irradiation duration per application site was 10 sec (70 sec per leg), resulting in an energy per point of 0.6 J and a total energy per leg per session of 25.2 J (total of 50.4 J). Each laser emitter had a power density of 21.43 W/cm², therefore delivering an energy density (fluence) of 214.28 J/cm² per irradiated point. These irradiation parameters were the same used previously by Ferraresi et al.¹⁷

Experimental protocol

Evaluation period. The exercise protocol was performed in a standardized manner. The volunteers performed the exercises in a seated position and in the same pattern at the same time of day (because of the effects of the circadian rhythm). The exercise and samples collection were repeated during two separate sessions (days 1 and 8), which were held on the same day of the week (Monday) and at the same time of day (8:30–11:30 a.m.). No strenuous physical activity was

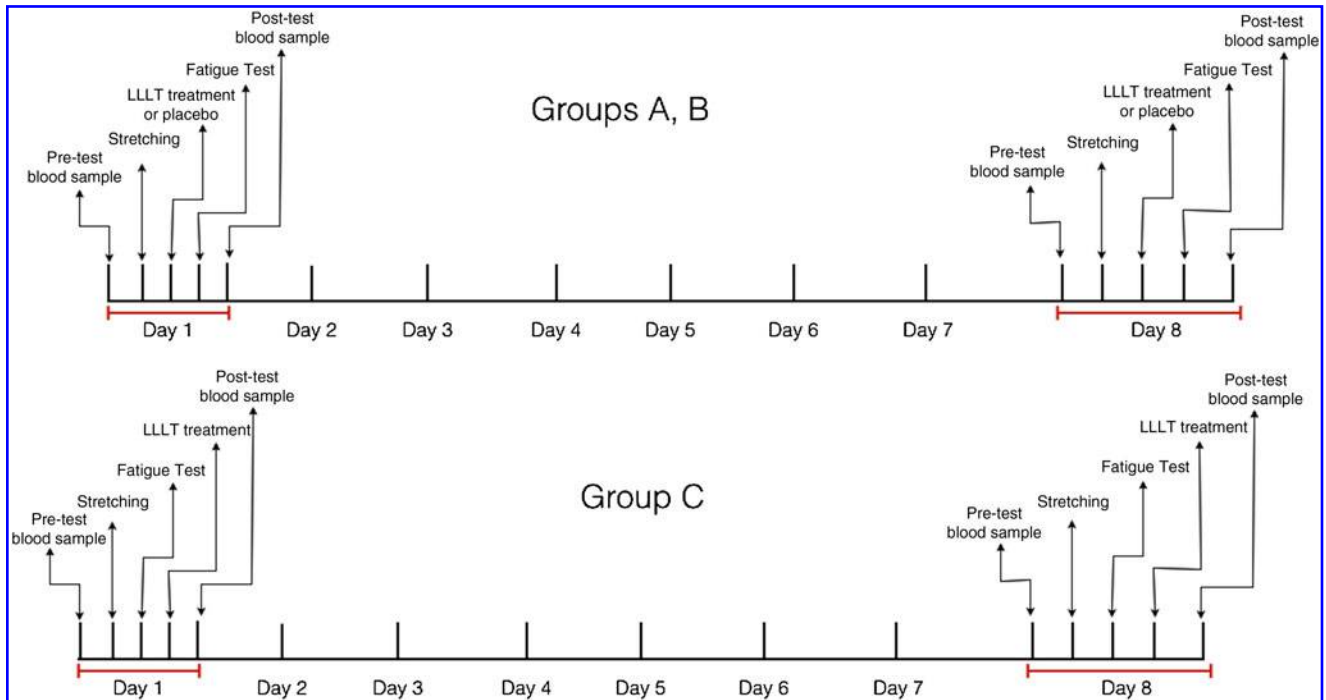


FIG. 1. Timeline for the period of evaluation of groups A, B, and C.

permitted during the weekend before the test. As we aimed to investigate acute effects of LLLT in skeletal muscle fatigue and biochemical markers related to recovery, we chose to use the same time procedures regarding evaluation period described by Leal Junior et al. in two studies.^{1,14} In both studies,

the authors showed that the study design was not affected by aspects such as residual effects of LLLT in the first session or familiarization with exercise protocol. The experimental protocol and timeline are summarized in Fig. 1.



FIG. 2. Applicable points of low-level laser therapy (LLLT) in quadriceps muscle.

Fatigue protocol. In each session (days 1 and 8) of the study, the baseline lactate and CK concentrations were measured first in each volunteer. Immediately afterward, all 27 volunteers were subjected to a series of stretching exercises involving all major muscles of the lower limbs (two sets of 60 sec for each muscle group).

Then, each volunteer was positioned in knee extension with the knee and hip flexed at 90 degrees. Using the weight load free of knee extension, each subject was tested to determine the maximum load (1 RM). Then, the subject was instructed to perform a full extension of the knee (90 to 0 degrees) at 75% of the maximum load. Failure to extend the knee fully (to 0 degrees) was recorded as quadriceps muscle fatigue. A goniometer was connected during leg extension to measure the angle of extension. The number of repetitions of the exercise fatigue test performed was counted by an observer, and the total time to complete the effort was measured by a second observer. The two observers were blinded to the participants' group allocations (A, B, or C). Repetitions began in the position of knee flexion (90 degrees) and ended in maximum extension (0 degrees).

Blood samples and analysis of the creatine kinase (CK) level and lactate concentration. To measure the serum CK levels, the ventral side of the dominant arm was cleaned and blood samples were collected aseptically. All blood collection procedures were performed by a qualified nurse (who was blinded to the group allocation). The samples were collected before and 5 min after the fatigue protocol. The blood was analyzed in the laboratory using infrared

TABLE 1. MEAN VALUES \pm STANDARD DEVIATION OF THE NUMBER OF REPETITIONS, TIME AND RM 75% FOR THE GROUPS AT DIFFERENT MOMENTS ($N=27$)

	Placebo	Pre-fatigue laser	Post-fatigue laser	p
<i>First session (day 1)</i>				
Number of repeats	39.9 \pm 17.1	31.0 \pm 11.2	28.7 \pm 8.9	0.1704 ^a
Time (sec)	41.1 \pm 14.7	36.0 \pm 9.2	34.2 \pm 7.9	0.3996 ^a
RM 75%	40.0 \pm 10.3	40.6 \pm 10.7	39.1 \pm 11.3	0.9764 ^a
<i>Second session (day 8)</i>				
Number of repeats	41.2 \pm 18.2	37.8 \pm 13.1	41.6 \pm 17.4	0.8965 ^a
Time (sec)	40.4 \pm 14.8	37.4 \pm 9.6	37.8 \pm 10.6	0.8424 ^a
RM 75%	41.7 \pm 9.7	41.7 \pm 13.7	42.2 \pm 6.7	0.9915 ^b

^aANOVA; ^bKruskal-Wallis.

spectrophotometry. Blood samples of lactate were collected aseptically from the cleaned second finger of the dominant arm. The samples were collected using the soft Accu-Check (Clix[®]) apparatus and analyzed immediately using a portable lactate analyzer (Accutrend[®]), before and 5, 10, and 15 min after the fatigue protocol.

Statistical analysis

The measured variables were expressed as the mean \pm standard deviation. The results were tabulated in order to examine the distribution of the values of the variables among the various groups. Between-group comparisons were analyzed using analysis of variance (one way ANOVA) with the Tukey post-hoc test for the parameters with normally distributed values and the Kruskal-Wallis test with Dunn's post-hoc test for the parameters with non-normally distributed values. The normality was tested using the Shapiro-Wilk test. A p value of ≤ 0.05 was considered to indicate statistical significance. The data were organized using Microsoft Office Excel 2010, and statistical analysis was performed using GraphPad Prism 5.0.

Results

The number of repetitions performed in each session (days 1 and 8) did not differ significantly among the groups. Although the number of repetitions increased between days 1 and 8 in the post-fatigue laser group but not in the placebo group, this difference was not significant. The time to fatigue

did not differ significantly among the groups during either session (days 1 and 8), and the values were very similar among the groups. Likewise, the variable RM 75% did not differ significantly among the three groups (Table 1).

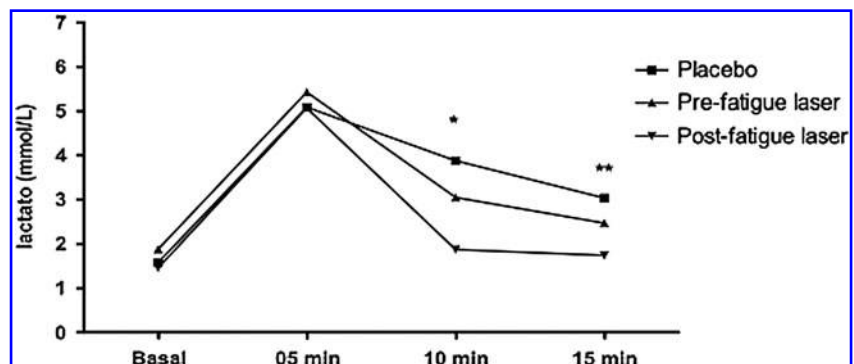
The lactate levels are shown in Fig. 3. Intergroup analysis showed significant reductions in the lactate levels 10 and 15 min after fatigue in the post-fatigue laser group versus the placebo group ($p < 0.01$ and $p < 0.05$, respectively) during the first session (day 1). There was also a reduction in the lactate level 15 min after fatigue in the post-fatigue laser group relative to the placebo group (Fig. 4) in the second session (day 8). Pre-fatigue laser treatment tended to reduce the lactate levels 10 and 15 min after fatigue, but these differences were not significant.

Intergroup analysis showed significant reductions in the CK concentration in the post-fatigue laser group relative to the placebo ($p < 0.01$) and pre-fatigue laser ($p < 0.05$) groups during the second session (day 8) (Fig. 5).

Discussion

The literature includes several studies in animals¹⁴ and humans¹⁷ that demonstrate positive effects of LLLT during the acute and chronic phases of inflammatory diseases.^{18,19} However, muscle fatigue and post-exercise recovery are new areas of LLLT research, and few studies have been conducted on this subject. To our knowledge, this is the first study to compare in the same experiment the effects of LLLT applied before and after exercise.

FIG. 3. Evolution of lactate values for the groups at baseline (day 1) as the analysis time (*Kruskal-Wallis, $p=0.0111$. Placebo versus post-fatigue laser: $p < 0.01$; **ANOVA, $p=0.0150$: placebo versus post-fatigue laser: $p < 0.05$).



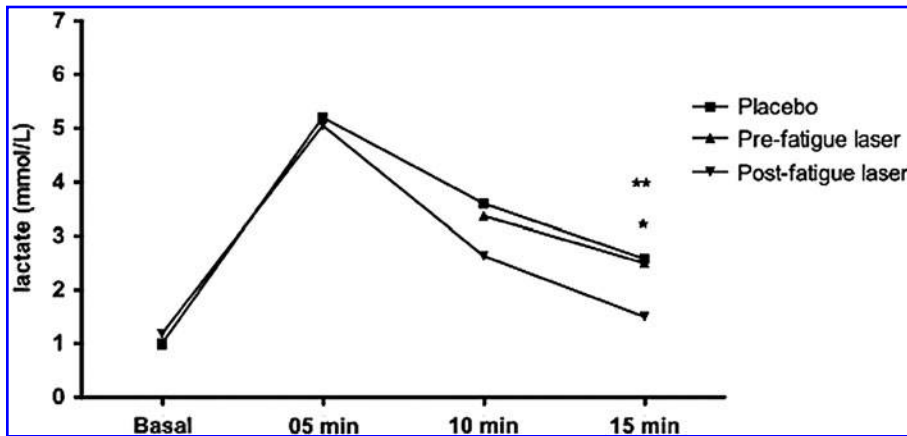


FIG. 4. Evolution of lactate values for groups at the second assessment as time analysis (*ANOVA, $p=0.0037$: placebo versus post-fatigue laser: $p<0.01$, **pre-fatigue laser versus post-fatigue laser: $p<0.05$).

The interaction of laser energy with biological tissues can trigger bioenergetic and proliferative effects on cellular and molecular levels. The cellular photoreceptors are located primarily in the mitochondrial respiratory chain,^{20,21} and laser treatment has accordingly been reported to increase the respiratory rate and mitochondrial adenosine triphosphate (ATP) synthesis.^{22,23} However, there is disagreement in the literature as to whether the laser is most effective when applied before or after muscle fatigue. There are reports that the bioenergetic effects are most pronounced after the tissue has been subjected to oxidative stress, as this increases the responsiveness of the receptors. Furthermore, animal studies²⁴ indicate that LLLT can act locally to prevent ischemic muscle damage by decreasing the activity of CK and the re-release of ROS, while increasing the levels of antioxidants and heat shock proteins.

The present study was performed to compare the effects of laser treatment among groups (placebo, pre-fatigue laser, and post-fatigue laser) in order to elucidate whether laser

therapy is more effective when applied before or after fatigue. We found that laser treatment decreased the serum CK and lactate levels versus placebo treatment in both groups, but that the effect was more significant in the post-fatigue laser group.

The optimal parameters for laser treatment (such as power, irradiation time, dose) are another topic of disagreement. The studies performed thus far do not support any conclusion as to the ideal conditions for reducing or delaying muscle fatigue, leaving a gap in our knowledge regarding the best "therapeutic window" for avoiding muscle fatigue.

With respect to the wavelength, the first important point is that irradiation with infrared laser (904 nm) produced a dose response similar to that for red laser (655 nm). This finding agrees well with the results of previous *in vivo*²⁵ and human²⁶ studies, and suggests that the anti-inflammatory effects of laser irradiation are not wavelength dependent. The use of longer wavelengths (808, 830, or 904 nm) has been suggested, because of the greater depth of penetration and absorption of the photons emitted by long-wavelength lasers.²⁷ In the present study, we used a wavelength of 830 nm to stimulate the quadriceps muscles, as longer wavelengths should be better absorbed by this deep muscle group.

Other methods of assessment used in this study were analysis of the kinetics of fatigue according to the levels of markers of biochemical (lactate) and muscle damage (CK). This randomized, double-blind, placebo-controlled study showed significantly lower levels of these markers, indicating reduction in exercise-induced fatigue. We suggest that the improvements in performance provided by the physical action of LLLT resulted from the lower activity of CK, increased levels of antioxidants, and improved microcirculation and lactate removal.

Intergroup assessment of the marker lactate showed the expected increase 5 min post-exercise in all groups, and significant reductions in the post-fatigue laser group after 10 and 15 min. Analysis of the pre-fatigue laser group showed a trend toward reductions in the lactate level after 10 and 15 min, but these changes were not significant for the first or second session (days 1 and 8). These results demonstrate that laser application after fatigue is more effective for removing blood lactate.

The role of the lactate level in the development of skeletal muscle fatigue is controversial. However, acidity during

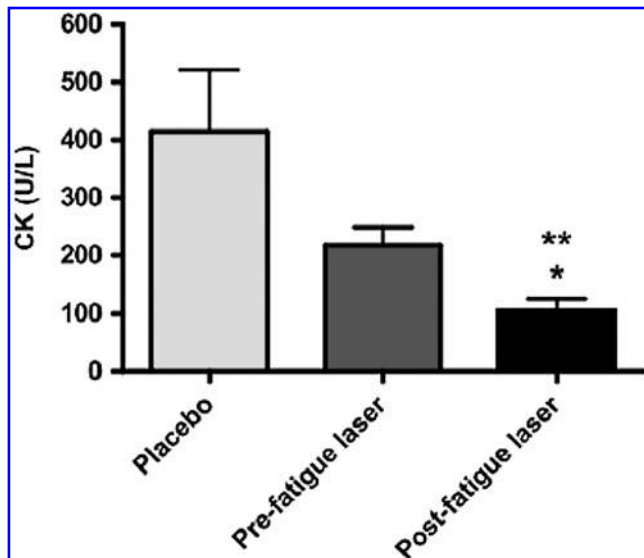


FIG. 5. Comparison of creatine kinase after exercise between the various groups in the second session (day 8) (Kruskal-Wallis; $p=0.004$). *Pre-fatigue laser versus post-fatigue laser $p<0.05$. **Placebo versus post-fatigue laser $p<0.01$.

excitation-contraction coupling can impair neuromuscular transmission, and hence the ability of muscle to contract. Some studies^{28–30} have shown that increasing the concentration of H⁺ inhibits the binding of Ca²⁺ to troponin, and can, therefore, affect the interaction between the contractile proteins.

Several factors could be responsible for the inhibition of the expected post-contraction increase in blood lactate levels in all irradiated groups. As laser irradiation can improve microcirculation,³¹ another possible mechanism of the reduction in the lactate concentration by laser irradiation may be related to the prevention of muscle ischemia at the cellular level.

In our study, we observed reductions in the levels of CK in the pre-fatigue and post-fatigue laser groups, but the reduction was significant for the post-fatigue laser group only during the second session (day 8). The decrease in CK activity after active LLLT may be related to the protective effect of laser irradiation against the development of muscle ischemia.

These effects may, in turn, contribute to the decreased activity of CK and delayed development of fatigue observed in the present study.

In addition to the previous hypotheses, the present study explored three other possible physiological mechanisms of the improvement of human exercise performance by LLLT. All are based on the importance of mitochondria in cellular energy production.

The present study has certain limitations, including the lack of evaluation of the blood for immune and inflammatory responses or metabolic enzymes such as interleukins 1- α and 6. Another limitation is that the irradiation time per point/row was very small (10sec) and this could decrease effects of LLLT both pre- and post-exercise. In the same way, temperature of the body, especially in the quadriceps area and the lower body in general, was not assessed for possible changes after laser therapy; however, the laser-treated volunteers did not report any sensation of warming.

Because this is a new area of research, more studies are needed to elucidate the ability of LLLT to delay muscle fatigue, as well as to define the "therapeutic windows" for the application of LLLT for treatment of different clinical conditions (e.g., fatigue, muscle damage, and fibromyalgia).

Conclusions

Laser treatment significantly reduced the serum lactate levels (in the pre-fatigue and post-fatigue laser groups) measured 10 and 15 min after exercise, and also reduced the level of CK in the post-fatigue laser group only, indicating that LLLT can be effective for improving muscle performance.

Author Disclosure Statement

Professor Ernesto Cesar Pinto Leal-Junior receives research support from Multi Radiance Medical (Solon, OH, USA), a laser device manufacturer. Multi Radiance Medical had no role in the planning of this study, and the laser device used was not theirs. They had no influence on study design, data collection and analysis, decision to publish, or preparation of the article. The remaining authors declare that they have no conflict of interests.

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