Effects of Low-Level Laser Therapy (LLLT) in the Development of Exercise-Induced Skeletal Muscle Fatigue and Changes in Biochemical Markers Related to Postexercise Recovery

Physical therapists use a variety of electrophysical agents. In some instances, these electrophysical agents are used to enhance the recovery between training sessions, to prevent sports injuries and, consequently, improve an athlete’s performance. Studies investigating the effects of commonly used interventions, such as massage, low-intensity exercises, cryotherapy, hot-cold contrast baths, neuro-muscular electrical stimulation, and stretching, are few, and the results on effectiveness are mixed. The rationale behind the use of these interventions is often related to mechanisms such as reducing postexercise inflammatory responses and the promotion of circulation and local metabolism.

- **STUDY DESIGN:** Randomized crossover double-blinded placebo-controlled trial.
- **OBJECTIVE:** To investigate if low-level laser therapy (LLLT) can affect biceps muscle performance, fatigue development, and biochemical markers of postexercise recovery.
- **BACKGROUND:** Cell and animal studies have suggested that LLLT can reduce oxidative stress and inflammatory responses in muscle tissue. But it remains uncertain whether these findings can translate into humans in sport and exercise situations.
- **METHODS:** Nine healthy male volleyball players participated in the study. They received either active LLLT (cluster probe with 5 laser diodes; $\lambda = 630$ nm; 200 mW power output; 30 seconds of irradiation, applied in 2 locations over the biceps of the nondominant arm; 60 J of total energy) or placebo LLLT using an identical cluster probe. The intervention or placebo were applied 3 minutes before the performance of exercise. All subjects performed voluntary elbow flexion repetitions with a workload of 75% of their maximal voluntary contraction force until exhaustion.
- **RESULTS:** Active LLLT increased the number of repetitions by 14.5% (mean $\pm$ 50.396 ± 4.3 versus 34.6 ± 5.6; $P = .037$) and the elapsed time before exhaustion by 8.0% ($P = .034$), when compared to the placebo treatment. The biochemical markers also indicated that recovery may be positively affected by LLLT, as indicated by postexercise blood lactate levels ($P < .01$), creatine kinase activity ($P = .017$), and C-reactive protein levels ($P = .047$), showing a faster recovery with LLLT application prior to the exercise.
- **CONCLUSION:** We conclude that pre-exercise irradiation of the biceps with an LLLT dose of 6 J per application location, applied in 2 locations, increased endurance for repeated elbow flexion against resistance and decreased postexercise levels of blood lactate, creatine kinase, and C-reactive protein.
- **KEY WORDS:** biceps, skeletal muscle damage, skeletal muscle performance

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for drainage of fluids and metabolites. Unfortunately, the available studies have methodological limitations, such as the inclusion of untrained subjects, small numbers of participants, and the use of surrogate outcomes. These limitations hamper generalization of the available trial results.

Neuromuscular electrical stimulation is an intervention that has been tested in postexercise recovery for soccer and futsal athletes. No significant differences were found for biochemical markers or performance outcomes after electrical stimulation compared to other interventions such as water and dry-land exercises and control (passive rest recovery) conditions. However, significant improvements were found for the subjective outcomes of pain reduction and perceived benefit with electrical stimulation.

Light amplification by stimulated emission of radiation (laser) was developed in the 1960s, using light with special properties like monochromaticity and low divergence.

Low-level laser therapy (LLLT) is the application of light (usually a low-power laser in the range of 1 to 500 mW) to a pathology. The light is typically of narrow spectral width in the red or near infrared spectrum (600-1000 nm), with a power density or irradiance (power output divided by laser spot area) between 1 mW and 5 W/cm². Infrared wavelengths penetrate better through the human skin than red wavelengths, and for this reason, lasers with infrared wavelengths are much more commonly used in physiotherapy clinical practice. One of the possible mechanisms behind the therapeutic effects of LLLT is the interaction of photons with laser irradiation at optimal doses (therapeutic window) with specific receptors in the mitochondria. It increases mitochondrial function, ATP, RNA, and protein synthesis. This interaction leads to increased oxygen consumption and membrane potential and enhanced synthesis of NADH and ATP. It consequently increases the cellular metabolism, possibly increasing the wound healing and accelerating the inflammatory process.

LLLT has become popular with physical therapists in some countries like Norway and Brazil. During the first wave of interest in the use of LLLT for therapeutic benefits in the late 1980s a limited number of clinical studies were performed with mixed outcomes. Controversy remained and leading medical experts expressed skepticism over the method during the 1990s. By the turn of the century, a renewed interest led to a slowly emerging research activity that identified several potential mechanisms of action and related dose-response patterns.

Studies performed in animals have shown positive effects of LLLT in the form of inflammatory reduction and improvement in muscle repair when the optimal parameters of irradiation were used. Studies into the mechanisms behind these effects suggest that LLLT can decrease oxidative stress and reactive oxygen species production, improve mitochondrial function, and stimulate mitochondrial respiratory chain, ATP synthesis, and microcirculation. These effects provide the rationale for testing if LLLT can prevent the development of skeletal muscle fatigue and enhance recovery.

We have previously performed clinical studies with single-laser diode probes to test if LLLT could delay the development of skeletal muscle fatigue and increase muscle recovery when applied before exercise. In these studies, LLLT decreased muscle fatigue and improved biochemical markers related to muscle recovery. However, our results were limited by the use of single-laser diode probes, which limited the size of the area of irradiation. In contrast, cluster multidiode probes make it possible to irradiate several points at the same time. This could increase the effects of LLLT, especially when large areas need to be irradiated such as skeletal muscles.

With this perspective in mind, we investigated whether LLLT, performed with a cluster multidiode probe over the biceps pre-exercise, would increase the number of submaximal repetitions of elbow flexion performed before exhaustion and reduce the level of the biochemical markers related to skeletal muscle recovery in top-level athletes.

**METHODS**

The study was designed as a crossover, randomized, double-blinded, placebo-controlled trial. All subjects signed a written declaration of informed consent and their rights were protected. The volunteers were recruited among male volleyball players of a single team competing at the highest national competitive level. The protocol for this study was approved by Vale do Paraiba University Research Ethics Committee.

**Randomization and Blinding Procedures**

Randomization was performed by a simple drawing of a card, which determined whether active LLLT or placebo LLLT should be given at the first exercise session. At the second session participants were crossed over to receive whichever treatment was not given at the first session. The code from the drawing was delivered to a technician who preset the treatment unit accordingly to either an active LLLT or placebo LLLT mode. The technician was also instructed not to communicate the type of treatment given to either the participants, the therapist applying the laser treatment to the biceps, or the observers. Thus the allocation of treatment was concealed to participants, therapist, and observers. Blinding of participants and the therapist was further maintained by the use of opaque goggles during LLLT procedures. The goggles also served to protect the eyes from LLLT irradiation.

**Inclusion/Exclusion Criteria**

Healthy male volleyball players, aged between 18 and 20 years, who had been...
Playing volleyball at the national level for at least the past 3 years, were included in the study. Exclusion criteria consisted of any previous musculoskeletal injury to the shoulder or elbow region, participation in less than 80% of the scheduled team physical training and volleyball sessions for the previous 3 months, and the use of any kind of nutritional supplements or pharmacological agents.

Nine athletes met the inclusion and exclusion criteria and were included in the trial (FIGURE 1).

**Procedures**

To provide a standard testing condition for the elbow, we used a Scott exercise bench, with an inclination angle of 45°. For the measurements of irradiation time and total time of repetitions, a Casio chronometer precise to 1/100 of a second was used.

**Maximum Voluntary Contraction (MVC) Test** Athletes were familiarized to the performance of elbow flexion-extension exercises (nondominant arm) with an adaptation period of 2 weeks. This consisted of performing 3 sets of 15 repetitions with a load equal to 7.5% of the athletes’ body weight during the team’s regular strength training sessions (3 times per week). After 2 weeks of familiarization with the exercise, we performed an MVC test (or 1-repetition maximum test) that consisted of establishing the largest load that could be lifted for a single repetition of elbow flexion from full extension to 90° of flexion for the nondominant elbow. The test was performed with the subject seated on a Scott bench (to control positioning and provide stabilization). Free weights (dumbbells) were used. After determining the MVC, the specific individual weight (load) corresponding to 75% of MVC was calculated for each subject.

**Period of Evaluation** Care was taken to standardize the exercise protocols and testing sessions. Exercises were performed in a standardized sitting position, and testing was performed in 2 separate sessions 7 days apart, such that both ses-

**FIGURE 1. CONSORT flow chart.**
sions were performed on the same day of the week (Monday) and the same time of day (between 4:30 and 8:30 PM). The first testing session was performed 2 days after the MVC test. High-level physical activity, such as game matches, strength training, or volleyball training sessions, was not allowed in the weekend before testing.

Fatigue Protocol At the beginning of each testing session, baseline blood measurements were obtained for each subject from the ventral side of the nondominant arm. This was immediately followed by a series of muscle-stretching exercises involving all the major muscles of the nondominant arm (2 repetitions of 60 seconds for each muscle group), finishing with the flexor muscles of the nondominant elbow. Then the subject was seated on the Scott bench, with the knees and hips flexed at 90°. Using free weights, the previously defined individual load corresponding to 75% of MVC was used for each subject. Using their nondominant arm, the subjects were instructed to perform repeated elbow flexion-extension from full elbow extension to 90° of flexion at maximal speed. A goniometer was fixed to the Scott bench to monitor the elbow flexion angle. The number of repetitions performed until fatigue was counted by 1 observer, and the total time to fatigue was measured by a second observer (FIGURE 2). The exercise protocol was considered complete when the subject did not reach the elbow flexion of 90°. During the execution of exercise protocol, the subjects received verbal encouragement provided by the observer who measured time to fatigue.

LLL T Procedure At each testing session, the participants either received a single treatment of active cluster LLLT or placebo cluster LLLT, both using a cluster with 5 laser diodes of 810 nm (THOR Photomedicine Ltd, Chesham, UK). The treatment sequence was randomized. The active or placebo LLLT was administered immediately after the stretching exercises and 3 minutes before the exercise fatigue test. Two irradiation sites evenly distributed in the middle of the ventral aspect of the biceps muscle (nondominant arm) were selected (FIGURE 3).

The LLLT irradiation was performed with the probe in direct contact with the skin, applying slight pressure, and with the probe held stationary oriented perpendicular to the skin. The parameters for the cluster probe LLLT (active and placebo) are summarized in TABLE 1.

After application of the active or placebo LLLT, participants were immediately repositioned, then started to perform the repeated-elbow-flexion protocol. The interval between application of the active or placebo LLLT and starting the testing was 180 seconds.

Blood Samples Possible muscle damage and inflammatory response were indirectly measured by creatine kinase (CK) activity and C-reactive protein (CRP) levels, respectively. To measure those parameters, a qualified nurse blinded to group allocation performed aseptic cleaning of the ventral side of the nondominant arm and took 1 blood sample before the stretching and laser or placebo treatments and another blood sample exactly 5 minutes after the exercises were completed. The samples were frozen, and blood analysis for CK was performed 1 week later using an infrared spectrophotometer (FEMTO Indústria e Comércio de Instrumentos, São Paulo, SP, Brazil) and specific analysis kit (Labtest Diagnostica SA, Lagoa Santa, Brazil). The analysis of CRP was also performed at that time by the agglutination method using a specific analysis kit (Wiener Laboratorios SAIC, Rosario, Argentina). All blood analyses were performed by an observer who was

TABLE 1

<table>
<thead>
<tr>
<th>Parameters for Cluster Low-Level Laser Therapy</th>
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<tbody>
<tr>
<td>Number of laser diodes</td>
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<td>Wavelength</td>
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<td>Frequency</td>
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<td>Optical output</td>
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<td>Spot size</td>
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<tr>
<td>Treatment time</td>
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<td>Number of irradiation points per muscle</td>
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<tr>
<td>Total energy delivered per muscle</td>
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<tr>
<td>Application mode</td>
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Blinded to the laser or placebo treatment allocations.

**Blood Samples for Blood Lactate Analysis**

To measure blood lactate concentrations, we took blood samples after aseptic cleaning of the second finger of the nondominant arm. The procedure was performed by a qualified nurse (blinded to group allocation), who took 1 sample before stretching and laser or placebo treatments, and additional samples at 5, 10, 15, and 20 minutes after the exercises were completed. The Accu-Chek Soft Clix lancets were used, and the samples were immediately analyzed with the portable Accutrend Lactate analyzer. The observer that performed the blood lactate analyses was also blinded to the laser or placebo treatment allocations.

**Statistical Analysis**

Group means and their respective standard deviations were used for statistical analysis. To analyze if a carryover effect occurred between the 2 exercise sessions, a 2-sided unpaired t test was used to compare the number of resisted elbow flexion repetitions performed and the time to perform these repetitions. A 2-sided paired t test was used to test if there was a significant difference for the number of resisted elbow flexion repetitions performed, time used to perform these repetitions, CK activity, and CRP levels between treatment using the active cluster LLLT and the placebo LLLT. A mixed-design analysis of variance (ANOVA) with Tukey-Kramer posttest was used to determine if there was a significant difference in blood lactate levels between treatment using the active cluster LLLT and placebo cluster LLLT. All statistical analyses were performed using GraphPad InStat Version 3.00 for Windows (GraphPad Software, San Diego, CA). The significance level was set at $P<.05$.

**RESULTS**

Nine healthy male volleyball players were recruited, who met the inclusion criteria. Their average age ± SD was 18.6 ± 1.0 years, their body mass 83.6 ± 5.60 kg, and their height 193.3 ± 8.8 cm.

In the analyses of possible crossover and unintended learning effects between testing sessions, the number of elbow flexion repetitions in the placebo and LLLT groups was compared using an unpaired t-test. The results showed a significant difference between the two groups ($P = .037$), indicating that the LLLT group performed more repetitions than the placebo group. Additionally, the time to perform the repetitions was analyzed using a mixed-design ANOVA, with significant differences found between the groups ($P = .004$).

**TABLE 2**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Active LLLT Exercise Session 1</th>
<th>Placebo LLLT Exercise Session 1</th>
<th>Active LLLT Exercise Session 2</th>
<th>Placebo LLLT Exercise Session 2</th>
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<tr>
<td>A</td>
<td>39.8</td>
<td>35.9</td>
<td>40.9</td>
<td>44.5</td>
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<tr>
<td>B</td>
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<td>36.1</td>
<td>36.5</td>
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<tr>
<td>C</td>
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<td>34.4</td>
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<tr>
<td>D</td>
<td>47.5</td>
<td>43.5</td>
<td>38.8</td>
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<td>E</td>
<td>45.8</td>
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Mean ± SD: 40.2 ± 6.4; 38.7 ± 3.7

**TABLE 3**

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Mean ± SD: 40.2 ± 6.4; 38.7 ± 3.7

**Abbreviation:** LLLT, low-level laser therapy.

* No significant difference between subjects who had active LLLT at the first session versus the second session ($P = .5180$, unpaired t tests). No significant difference between subjects who had placebo LLLT at the first session versus the second session ($P = .5301$, unpaired t tests).
ion repetitions performed, and time to perform these repetitions were not affected ($P > .05$) by whether active LLLT was given at the first or last session (TABLES 2 and 3).

The mean number of resisted elbow flexion repetitions performed was 14.5% higher (mean ± SD, 39.6 ± 4.3 repetitions) when the volunteers received the active LLLT treatment before the exercise fatigue tests, compared to when they received the placebo LLLT (34.6 ± 5.6 repetitions, $P = .037$) (FIGURE 4).

The mean ± SD amount of time to perform the resisted elbow flexion exercise test was 8.0% longer after treatment with the active LLLT (41.3 ± 5.1 seconds) than after treatment with placebo LLLT (38.2 ± 3.2 seconds; $P = .034$) (FIGURE 5).

The subjects presented with similar blood lactate levels prior to laser (1.3 ± 0.1 mmol·L$^{-1}$) and placebo (1.4 ± 0.2 mmol·L$^{-1}$) treatment ($P > .05$).

The blood lactate levels increased in both groups from baseline assessments to postexercise assessments. There was a significant difference between the groups at 5 minutes postexercise (active LLLT, 2.2 ± 0.5 mmol·L$^{-1}$; placebo LLLT, 5.3 ± 3.2 mmol·L$^{-1}$; $P < .01$). However, no significant differences in blood lactate levels were found between groups at 10, 15, or 20 minutes postexercise (FIGURE 6).

CK activity before the exercise test was similar between groups (active LLLT, 281.0 ± 196.3 U·L$^{-1}$; placebo LLLT, 340.6 ± 335.6 U·L$^{-1}$; $P > .05$). Postexercise CK activity was reduced after treatment with active LLLT (263.6 ± 134.2 U·L$^{-1}$) while it increased after treatment with placebo LLLT (525.7 ± 386.5 U·L$^{-1}$). This difference between treatments was significant ($P = .017$) (FIGURE 7).

CRP levels before the exercise test were similar between groups (active LLLT, 38.7 ± 44.0 mg·dl$^{-1}$; placebo LLLT, 26.7 ± 29.3 mg·dl$^{-1}$; $P > .05$). Postexercise CRP levels decreased after treatment with active LLLT (1.3 ± 4.0 mg·dl$^{-1}$), while it increased after treatment with placebo LLLT (92.0 ± 115.1 mg·dl$^{-1}$). This difference between treatments was significant ($P = .047$) (FIGURE 8).

**DISCUSSION**

In this study, we evaluated if the use of LLLT could affect the development of skeletal muscle fatigue and biochemical markers of skeletal muscle recovery. A robust study design was used, with all subjects receiving the active and the placebo treatment on 2 separate occasions and all investigators and subjects being blinded to the treatment received on each occasion. All procedures were also rigorously followed. The similarity of the group data at baseline prior to the 2 treatment options, along with the absence of a treatment order/learning effect, provides confidence in the results of this study.

Irradiation of the biceps muscle with active LLLT prior to repeated resisted elbow flexion significantly increased the number of repetitions before exhaustion, when compared to irradiation with placebo LLLT. Accordingly, increased
aimed at improving the LLLT treatment procedure. For this reason we treated a larger area using an applicator with 5 laser diodes and applied irradiation in 2 locations of the muscle belly. It is possible that 4 irradiation points, as used in previous studies, might have been insufficient to cover the biceps muscle and that increasing the treatment area to a total of 10 irradiation points, as done in this study, was the source of difference between the trials. Treatment dosage warrants attention in future studies, as it is possibly an important variable for LLLT administration. Because of the poor skin penetration ability of the lasers, a single diode will only cover a small area (2–3 cm²). Some authors have tried without success to overcome the poor distribution of laser light by introducing scanning laser devices. Nearly all basic science studies on LLLT have been performed with stationary treatment, and the general interpretation of published data suggest that LLLT is not effective if the laser source is not kept stationary over the same location for at least 20 to 30 seconds.

CK activity after exercise was significantly decreased in subjects who received the active LLLT. This result, together with the concurrent decrease in CRP levels, indicates a possible protective effect against exercise-induced muscle damage. These findings are consistent with a number of animal studies in which LLLT was found to reduce inflammation induced by inflammatory agents or trauma.

Surprisingly, CRP levels and CK activity were significantly lower after the exercises when compared to their pre-exercise values, after receiving the LLLT treatment. The decrease in CK activity and CRP levels after active LLLT could be related to a laser-protective effect in the development of muscle ischemia. There are some indications that LLLT can reduce reactive oxygen species release and creatine phosphokinase activity, while levels of antioxidants and heat shock proteins increase. In a muscle cell study, LLLT improved mitochondrial function and reversed a dysfunctional state induced by electrical stimulation.

Blood lactate concentration is widely used to monitor performance and recovery, and it is also a surrogate marker of recovery after exercise. Our findings indicate that treating the area with LLLT prior to the exercise reduces postexercise blood lactate levels at 5 minutes after exercise and possibly has a positive influence on recovery. Commonly used modalities to help recovery, such as massage, cryotherapy (cold-water immersion), and electrical stimulation have failed to significantly enhance blood lactate removal. Positive effects in blood lactate removal have been demonstrated after hot-cold baths but only in nonathletes. A reduction of postexercise blood lactate levels is desirable, because high lactate levels decrease the interstitial H⁺ concentration and intracellular pH, leading to acidosis. Acidification of the neuromuscular junction may impair the neuromuscular transmission and, consequently, muscle contractions. In addition, some studies have indicated that high H⁺ concentrations can inhibit the linking of Ca²⁺ to troponin and thereby inhibit the interaction between contractile proteins.

Dose and treatment procedure seem to be important to achieve positive effects of LLLT in muscle tissue. In previous studies we had irradiated the biceps muscle at 4 locations using a single-diode laser but failed to find significant differences in blood lactate levels compared to controls. In the current protocol, we
ous studies have also demonstrated that LLLT can stimulate the mitochondrial respiratory chain and ATP synthesis. Such effects could, in turn, contribute to a decrease in CK activity, CRP levels, and also the delay in development of fatigue seen in the current study. Some evidence suggests that other therapies such as massage and hot-cold water baths may prevent muscle damage after exercise. But cryotherapy (cold water immersion) did not decrease postexercise levels of biochemical markers of muscle damage or inflammation in previous studies.

In the management of muscle recovery among athletes, a multitude of interventions are commonly used, despite limited evidence to support their effectiveness. This current study builds on several earlier studies using LLLT performed on animals and humans. In earlier studies we first tried to elucidate the mechanisms involved in LLLT irradiation and their respective dose-response patterns. We used this knowledge to develop an LLLT treatment procedure that seemed optimal, which was the one tested in this latest study. But several questions remain unanswered, such as when to irradiate for best results and whether LLLT can improve subsequent performances when repeated participation is needed. LLLT dose recommendations have already been developed by the World Association of Laser Therapy for the treatment of tendons and joints. The World Association of Laser Therapy also recommends that doses in clinical studies should be calculated in Joules (J) only. Our dose measured in J/cm² may seem larger than doses used in other studies, but this is due to the very small spot size for the laser we used. Small spot sizes inflate the J/cm² dosage calculations and cause confusion. In humans, the target tissue is typically much larger than the laser spot size. The reasoning behind World Association of Laser Therapy guidelines is that it is incorrect to state clinical doses in J/cm² when only a small part of the surface of the target tissue is being irradiated. Consequently, the J/cm² should be limited to cell and animal studies, in which the target area can be fully covered. The main parameter for clinical doses in human studies should be Joules. More studies are needed to define the therapeutic window for muscle fatigue and damage, as well as muscle recovery.

**CONCLUSIONS**

A dose of LLLT (λ = 810 nm, 200 mW, 30 seconds, 164.85 J/cm², 6 J per point), administered to each of 10 treatment areas over the biceps muscle, significantly delayed the development of muscle fatigue during a task of repetitive resisted elbow flexion. This finding was consistent with observed changes in biochemical markers related to skeletal muscle recovery. This suggests that LLLT may have a protective effect on the development of muscle ischemia and exercise-induced muscle damage. Further studies are needed to find the optimal timing of LLLT irradiation for recovery, and if LLLT can improve physical performance during recovery or reduce the recovery period.

**KEY POINTS**

**FINDINGS:** This study showed that LLLT delayed the development of skeletal muscle fatigue and concurrently decreased postexercise levels of biochemical markers of muscle recovery.

**IMPLICATION:** These findings suggest that LLLT applied pre-exercise may be helpful to delay fatigue during a repetitive task and possibly help recovery.

**CAUTION:** The design of the experimental procedure using a single muscle group also has limitations, and the observed LLLT effects may not translate into more complex sporting activities involving several muscles.

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