Light-emitting diode therapy (LEDT) before matches prevents increase in creatine kinase with a light dose response in volleyball players
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Cleber Ferraresi · Ricardo Vinicius dos Santos · Guilherme Marques · Marcelo Zangrande · Roberley Leonaldo · Michael R. Hamblin · Vanderlei Salvador Bagnato · Nivaldo Antonio Parizotto

Abstract Low-level laser (light) therapy (LLLT) has been applied over skeletal muscles before intense exercise (muscular pre-conditioning) in order to reduce fatigue and muscle damage (measured by creatine kinase, CK) in clinical trials. However, previous exercise protocols do not exactly simulate the real muscle demand required in sports. For this reason, the aim of this randomized and double-blind placebo-controlled trial was to investigate whether light-emitting diode therapy (LEDT) applied over the quadriceps femoris muscles, hamstrings, and triceps surae of volleyball players before official matches could prevent muscle damage (CK) with a light dose response, establishing a therapeutic window. A professional male volleyball team (12 athletes) was enrolled in this study, and LEDT was applied before 4 matches during a national championship. LEDT used an array of 200 light-emitting diodes (LEDs) arranged in 25 clusters of 4 infrared LEDs (850±20 nm; 130 mW) and 25 clusters of 4 red LEDs (630±10 nm; 80 mW). Athletes were randomized to receive one of four different total doses over each muscle group in a double-blind protocol: 105 J (20 s), 210 J (40 s), 315 J (60 s), and placebo (no light for 30 s). CK in blood was assessed 1 h before and 24 h after each match. LEDT at 210 J avoided significant increases in CK (+10 %; \(P=0.993\)) as well as 315 J (+31 %; \(P=0.407\)). Placebo (0 J) allowed a significant increase in CK (+53 %; \(P=0.012\)) as well as LEDT at 105 J (+59 %; \(P=0.001\)). LEDT prevented significant increases of CK in blood in athletes when applied before official matches with a light dose response of 210–315 J, suggesting athletes might consider applying LEDT before competition.

Keywords Photobiomodulation · LLLT · CK · Muscle damage · Exercise recovery · Muscle performance

Introduction

The benefits of low-level laser (light) therapy (LLLT) to treat pain [1, 2], tendinopathies [3] and to promote tissue healing [2, 4] have been investigated for several years. The mechanism of the light-tissue interaction is thought to involve cytochrome c oxidase (Cox) as the main chromophore in the cells...
able to absorb specific wavelengths of light [5–9]. Cox is a mitochondrial enzyme with a very important function in the electron transport chain and consequently promotes cell respiration and energy production in the form of adenosine triphosphate (ATP). For these reasons, LLLT has been widely used for several types of medical treatment, especially those indications that require stimulation of cells and improved healing.

Recently, LLLT has been used to increase muscle performance [10, 11]. When applied after exercise, LLLT promoted reduction of fatigue [12] and increased the workload in maximum effort tests [13] after training programs. When applied before the exercise (muscular pre-conditioning), LLLT increased the number of repetitions and was able to promote “muscle protection” against exercise-induced muscle damage [10, 11] measured by a lower increase of creatine kinase (CK) levels in the blood. In this context, it is valuable to highlight that CK is an important enzyme of the energy metabolism located inside muscle cells, but its increase in the bloodstream after a bout of exercise is an indicative of rupture of muscle cell membrane and consequently muscle damage [14].

In order to investigate the effects of LLLT on “muscle protection” against exercise-induced muscle damage, different protocols of exercise or neuromuscular electrical stimulation have been used to induce muscle fatigue and damage in experimental models [15–20] and in clinical trials [21–28]. These studies have evaluated different wavelengths and different light sources such as diode lasers and light-emitting diodes (LEDs) [29]. Three recent studies [10, 11, 30] reported fascinating results and point to the effectiveness of muscular pre-conditioning using diode lasers and LEDs to prevent muscle fatigue and muscle damage (CK) when applied before (5 min) a bout of exercise.

Having in mind all previous results for muscular pre-conditioning [10, 11, 30], the present study aimed to investigate the effectiveness of the LLLLT by LED therapy (LEDT) in the prevention of muscle damage (CK) in professional volleyball players during a national championship. This randomized double-blind placebo-controlled study used an array of LEDs to irradiate equally all target muscle groups [13] with different doses of light in order to establish also a therapeutic window or dose response [31] for LEDT, thus translating these studies to clinical practice. Moreover, the LED array emitted red and infrared light at the same time based on studies that reported better absorption of the light by Cox using bandwidths in the red and near-infrared spectral regions [5–9]. The possible time response of 5 min widely reported in muscular pre-conditioning [10, 11, 30] was modified to 40–60 min, since previous studies already reported a range of 3–45 min for LLLT to increase ATP synthesis in cells [32, 33], and also made it possible to perform muscular pre-conditioning for all athletes before each official volleyball match.

Materials and methods

Study design and ethics statement

The present study was a randomized, double-blind, and placebo-controlled trial involving a professional team of volleyball players in the “Superliga” (national championship) in Brazil during four official matches. Each match was carried out in different stadiums, in accordance with the championship schedule. All researchers traveled with the coaching staff and volleyball players during the study. This study was conducted in compliance with the Declaration of Helsinki (1964) and its later amendments, and also approved by the Research Ethics Committee for Human Studies of the Federal University of Sao Carlos (number protocol approved 217/2012).

Volunteers

Twelve professional volleyball players (the whole team) were enrolled in the study. They had an average age of $25.5\pm5.3$ years old, body weight of $90.6\pm7.3$ kg, and height of $200\pm8.7$ cm. After their agreement, all players signed the informed consent statement.

Inclusion criteria and exclusion criteria

Inclusion criterion used was healthy professional volleyball players. Exclusion criterion used was volleyball players having musculoskeletal injuries prior to the study or injured during the course of the study.

Groups and randomization procedures

All athletes were randomly allocated into four different groups for muscular pre-conditioning in accordance with the assigned light dose (Joules, J) of the light-emitting diode therapy (LEDT):

- **Dose 1**—20 s of real LEDT (105 J total) over quadriceps femoris muscles, hamstrings, and triceps surae
- **Dose 2**—40 s of real LEDT (210 J total) over quadriceps femoris muscles, hamstrings, and triceps surae
- **Dose 3**—60 s of real LEDT (315 J total) over quadriceps femoris muscles, hamstrings, and triceps surae
- **Dose 4**—30 s of placebo LEDT (0 J total) over quadriceps femoris muscles, hamstrings, and triceps surae

Each athlete randomly received one of the light doses before each one of the four official matches of the championship. The randomization procedure was conducted at Randomization.com (http://www.randomization.com) using balanced permutations into one block with four different therapies: dose 1 to dose 4. The randomization procedure was carried out by evaluator #1 that operated...
the LED device. This researcher was instructed not to inform athletes, researchers, and coaching staff which dose was applied to each athlete at each match.

During the course of the study, two athletes suffered musculoskeletal injuries and then were excluded. One athlete belonged to dose 4 (30 s of irradiation—placebo) and the second athlete belonged to dose 2 (20 s of irradiation). However, these exclusions did not affect the number of subjects per group once all statistical analyses were performed using data of all the six principal active players (not reserve players) in each match.

**Experimental protocol**

*Light-emitting diode therapy*

LEDT used a flexible array of $34 \times 18$ cm ($612$ cm$^2$) similar to the one used in a previous study developed by our research group [34]. However, this prototype device has 200 LEDs arranged in 25 clusters of four infrared LEDs ($850\pm20$ nm; $130$ mW) and 25 clusters of four red LEDs ($630\pm10$ nm; 80 mW) displayed at five lines of ten clusters. Each line has one infrared cluster interspersed by one red cluster, totaling five infrared clusters plus five red clusters per line. Irradiation lasted 20, 40, 60, or 30 s (placebo) over each muscle group (quadriceps femoris, hamstrings, triceps surae) of each athlete’s leg with fixed parameters as described in Table 1. Real LEDT or placebo was applied between 40 and 60 min before the start of each official match, in accordance with randomization procedures. LEDT placebo had no energy (0 J) and no power (0 mW) applied over these muscle groups. Optical power was measured with an optical energy meter PM100D Thorlabs® fitted with a sensor S130C (area of 0.70 cm$^2$). All athletes were blinded for these therapies as well as the coaching staff and evaluator #2 until the end of the study. There was no perceptible sensation of heat to the skin from real LEDT.

**Blood samples for creatine kinase activity**

Blood samples for creatine kinase activity measurement were collected by puncturing the athlete’s ear lobe using sterile lancets 1 h before and 24 h after each official match. The puncture site was cleaned with alcohol and dried, and the first drop of blood was discarded. The blood collected (30 μL) was immediately analyzed with Reflotron Plus® biochemical analyzer (Roche, Germany) [14] following the manufacturer’s guidelines. This analysis was conducted by evaluator #2, and all results were blinded for evaluator #1, athletes, and coaching staff until the end of the study.

**Matches**

The time of each match was monitored by evaluator #3 (coaching staff) as well as which were the six active players in each match (not reserve players).

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**Table 1 Parameters of light-emitting diode therapy (LEDT)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of LEDs</td>
<td>200 (100 infrared-IR and 100 red-RED)</td>
</tr>
<tr>
<td>Wavelength</td>
<td>$850\pm20$ nm (IR) and $630\pm10$ nm (RED)</td>
</tr>
<tr>
<td>Number of clusters</td>
<td>25 (IR) and 25 (RED)</td>
</tr>
<tr>
<td>Frequency</td>
<td>Continuous output</td>
</tr>
<tr>
<td>Optical output (each cluster of 4 LEDs)</td>
<td>130 mW (IR) and 80 mW (RED)</td>
</tr>
<tr>
<td>Total optical output</td>
<td>$5250$ mW ($25\times130$ mW plus $25\times80$ mW)</td>
</tr>
<tr>
<td>LED spot size (cm$^2$)</td>
<td>0.2</td>
</tr>
<tr>
<td>Power density (each cluster)</td>
<td>$185.74$ mW/cm$^2$ (IR) e $114.28$ mW/cm$^2$ (RED)</td>
</tr>
<tr>
<td>Treatment time over each muscle group (s)</td>
<td>20, 40, or 60</td>
</tr>
<tr>
<td>Energy per cluster at 20 s</td>
<td>$2.6$ J (IR) and $1.6$ J (RED)</td>
</tr>
<tr>
<td>Energy per cluster at 40 s</td>
<td>$5.2$ J (IR) and $3.2$ J (RED)</td>
</tr>
<tr>
<td>Energy per cluster at 60 s</td>
<td>$7.8$ J (IR) and $4.8$ J (RED)</td>
</tr>
<tr>
<td>Energy density per cluster at 20 s</td>
<td>$3.71$ J/cm$^2$ (IR) and $2.28$ J/cm$^2$ (RED)</td>
</tr>
<tr>
<td>Energy density per cluster at 40 s</td>
<td>$7.42$ J/cm$^2$ (IR) and $4.56$ J/cm$^2$ (RED)</td>
</tr>
<tr>
<td>Energy density per cluster at 60 s</td>
<td>$11.13$ J/cm$^2$ (IR) and $6.84$ J/cm$^2$ (RED)</td>
</tr>
<tr>
<td>Total energy delivered per muscle group at 20 s</td>
<td>$105$ J [$2.6\times25=65$ J (IR) plus $1.6\times25=40$ J (RED)]</td>
</tr>
<tr>
<td>Total energy delivered per muscle group at 40 s</td>
<td>$210$ J [$5.2\times25=130$ J (IR) plus $3.2\times25=80$ J (RED)]</td>
</tr>
<tr>
<td>Total energy delivered per muscle group at 60 s</td>
<td>$315$ J [$7.8\times25=195$ J (IR) plus $4.8\times25=120$ J (RED)]</td>
</tr>
<tr>
<td>Total energy delivered on body (J)</td>
<td>630</td>
</tr>
<tr>
<td>Total energy delivered on body (J)</td>
<td>1260</td>
</tr>
<tr>
<td>Total energy delivered on body (J)</td>
<td>1890</td>
</tr>
</tbody>
</table>

Application mode: Device held coupled in skin contact
Statistical analysis

Although this study enrolled a whole team (12 athletes), and all athletes received one of the light doses before each match, the statistical analysis was applied to the six principal active players (not reserve players) in each match. Thus, each group (light dose) had 6 subjects, and the study had a total sample size of 24 subjects. Shapiro-Wilk’s W test verified the normality of the data distribution. Creatine kinase activity among all groups was compared using two-way analysis of variance (ANOVA) with repeated measures and Tukey honest significant difference (HSD) post hoc test. Significance was set at $P<0.05$.

Results

Matches

The average time of the matches was $133.75 \pm 8.99$ min.

Creatine kinase activity

All results of CK activity were presented as mean ± standard deviation (SD). LEDT dose 1 (20 s, 105 J) allowed a significant mean increase in CK (from 328.0±188.9 to 499.6±232.0 U/L; +59%; $P=0.001$). Dose 4 (30 s—placebo, 0 J) also allowed a significant increase in CK (from 270.3±112.4 to 406.1±150.5 U/L; +53%; $P=0.012$). However, LEDT dose 2 (40 s, 210 J) avoided a significant increase in CK (from 338.8±130.3 to 364.1±127.5 U/L; +10%; $P=0.993$). Dose 3 (60 s, 315 J) also prevented a significant increase in CK (from 245.1±126.9 to 318.0±153.5 U/L; +31%; $P=0.407$). These results were measured in all six principal active players of each match and presented in Fig. 1.

Discussion

This study investigated the dose response of light-emitting diode therapy to prevent significant increases in creatine kinase activity in volleyball players during official matches, establishing a “therapeutic window” (dose response of different doses of light). We applied LEDT on lower limb muscles aiming to cover the major muscle groups involved in jumping and landing movements that are required during volleyball matches. Moreover, the array of LEDs used in this study covered the entire target muscle groups as has been previously recommended by our research group [13]. To our knowledge, this is the first study that applied LEDT on muscles of professional athletes before official matches to prevent muscle damage.

The effects of low-level laser (light) therapy on muscle tissue when applied before or after intense exercise are mainly related to the prevention of exercise-induced damage, promotion of faster muscle recovery, and also producing increases in performance [10, 30, 11]. The use of LLLT to prevent muscle damage has been widely investigated in experimental models [15–20] and in clinical trials [21–28]. Experimental studies have used animal models to induce muscle damage, and clinical trials have used protocols of exercise in isokinetic dynamometers, fitness machines, or free weight lifting to induce muscle damage. However, all these studies could not exactly simulate the real muscular demand in athletes during official matches of any sport, motivating our research group to investigate the effectiveness of LEDT in volleyball players using a regimen of muscular pre-conditioning to prevent increases in CK.

Previous studies already reported benefits of the LLLT using diode lasers and LEDs to prevent increases in CK activity [10, 30, 11]. To our knowledge, the first study that used LLLT to prevent muscle damage was carried out by Lopes-Martins et al. [15] in an experimental animal model. These authors investigated the effects of different doses of light (0.5; 1.0 and 2.5 J/cm²) in muscular pre-conditioning to prevent muscle fatigue and muscle damage (CK) induced by neuro-muscular electrical stimulation. These authors reported a LLLT dose response to decrease CK activity in muscle tissue. Another experimental study trained rats on an inclined treadmill and measured inhibition of inflammation, reduction of CK activity, and lowering of oxidative stress (malondialdehyde, MDA). They also found increases in
defense against oxidative stress (increased activity of superoxide dismutase, SOD) 24 and 48 h after exercise [16]. These studies were important to demonstrate the benefits of LLLT on exercise-induced muscle damage, inflammation, and oxidative stress.

More studies using experimental models were conducted to investigate the effects of LLLT on CK activity [18, 17, 19, 20]. Using a similar model of neuromuscular electrical stimulation previously reported [15], other studies also found LLLT dose responses of muscular pre-conditioning by decreased CK activity [18, 19], including assessment of different wavelengths [20]. Another study observed a reduction in CK activity when LLLT was applied after exercises in a model of swimming with workload [17]. Recently, LLLT using LEDs (LEDT) has demonstrated similar benefits to decrease CK activity when applied after exercise [35] or during rest intervals between bouts of physical activity [36]. All these studies assessed CK activity after 24 h or during a time range of 24–48 h after the exercise.

These aforementioned experimental studies created the scientific basis for the use of the LLLT, including LEDT, in prospective clinical trials that aimed to prevent exercise-induced muscle damage by assessing CK activity. Recently, this effect of “muscle protection” by LLLT has been observed in humans using different exercises protocols for the upper and lower limbs [10, 11, 30]. These studies reported lower increases in CK activity measured in blood when LLLT was applied before intense exercises. Light doses (Joules, J) used in previous studies were between 1 and 6 J delivered per diode laser, totaling 4 J [21] or 60 J [22] delivered to the biceps brachii; 30 and 40 J [23] or 180 J [24] delivered to the quadriceps femoris muscles. When the light source was LED, these studies used a cluster of 69 LEDs and applied 0.3 or 0.9 J per LED, totaling 41.7 J per site of irradiation and a total dose of 41.7 J delivered on biceps brachii [25]; 83.4 J [26] or 125.1 J [27] or 208.5 J [28] delivered to quadriceps femoris muscles.

Our results are in accordance with the total dose used in previous studies that used LEDs (LEDT) or combined LEDs and super-pulsed lasers to prevent increases in CK activity in the quadriceps femoris muscles with total doses from 60 to 300 J [29]. Our effective total doses of LEDT were 210 J [130 J (IR) plus 80 J (RED)—40 s of irradiation] and 315 J [195 J (IR) plus 120 J (RED)—60 s of irradiation] as reported similarly by a previous study [29]. LEDT placebo and the total dose of 105 J [65 J (IR) plus 40 J (RED)—20 s of irradiation] allowed a significant increase of CK activity, indicating potential muscle damage and failure of the LEDT to promote “muscle protection.” In addition, we chose to use dual wavelengths (red and near-infrared at the same time) based on specific absorption bands of cytochrome c oxidase (Cox) [5–9] that is the main chromophore in the cells. Moreover, our results were observed using muscular pre-conditioning applied 40–60 min before the start of each official match. This consideration is important because the waiting time can allow the muscles more time to respond to the light and could change the accepted paradigm that muscular pre-conditioning using LLLT for the prevention of exercise-induced muscle damage should be applied 5 min before the exercise.

The optimum doses of light delivered from each cluster of four LEDs were between 5.2 and 7.8 J for infrared and between 3.2 and 4.8 J for red. These energy doses are similar to doses emitted by diode lasers in a previous study [24], reinforcing the idea that light is light [37] and there is not a large difference between these light sources to prevent exercise-induced muscle damage (CK) if the total dose applied per muscle group is adequate. However, it is important to remark that it is possible that the light dose used to prevent muscle damage with LEDT in a pre-conditioning regimen may not be the same dose necessary if the light therapy is applied after the exercise [38]. It is accepted that cells under biochemical or mechanical stress have better responses to light [39] than cells in homeostasis.

The number of jumps and landings or of any other movement performed by each athlete during each match was not equal and could not be standardized among the six principal active players. This could be understood as a limitation, but the course of each match was unpredictable and could not be standardized.

Finally, the present study was not designed to elucidate the mechanisms of action of LEDT when used in muscular pre-conditioning to prevent exercise-induced muscle damage. However, previous studies reported better defenses against oxidative stress [16] if LLLT is applied over muscles after intense exercises as well as before exercise as reported in a previous clinical trial [40]. As mentioned above, the main chromophore, or red/NIR light-sensitive protein, present in biological tissues is Cox [5]. After absorbance of light, mitochondrial metabolism is modulated, promoting increases in ATP synthesis as one of the secondary responses [5]. We suggest that “muscle protection” against exercise-induced damage by LLLT is also a secondary response, but the connection between the light absorption and this effect is not fully understood. As a suggestion for future studies, we believe that LLLT could possibly modulate the proteins of membrane channels in muscle cells and/or in proteins involved in the transduction of the mechanical stress generated during muscle contraction involving the extracellular matrix and cytoskeleton. These modulations could improve the biochemical environment and the mechanical response of the muscle cells to exercise by stabilizing the muscle cell membrane.

It should be noted that there is no current position taken by the World Anti-Doping Agency (WADA) or the International Olympic Committee (IOC) regarding the use of LLLT for the enhancement of muscle performance. However, if the use of LLLT before athletic competition becomes widespread, we...
expect discussions will have to take place in the appropriate circles on whether its use constitutes an unfair advantage.

Conclusion

Use of red and near-infrared LEDT for muscular pre-conditioning was effective in the prevention of muscle damage (CK) in professional volleyball players during official matches. There was a therapeutic window (dose response) for this effect with a better dose of 210–315 J applied over the entire target muscles than with 0 J (placebo) or 105 J. Our results will stimulate more researchers and teams of high-performance sports to use LEDT for the prevention of muscle damage in professional athletes.

Acknowledgments

Cleber Ferreira would like to thank FAPESP for his PhD scholarships (numbers 2010/07194-7 and 2012/05919-0). MR Hamblin was supported by US NIH grant R01AI050875.

Ethical statement

This study was conducted in compliance with the Declaration of Helsinki (1964) and its later amendments and also approved by the Research Ethics Committee for Human Studies of the Federal University of Sao Carlos (number protocol approved 217/2012).

Conflict of interest

The authors declare no conflict of interest.

References


