## Comparison Between Single-Diode Low-Level Laser Therapy (LLLT) and LED Multi-Diode (Cluster) Therapy (LEDT) Applications Before High-Intensity Exercise

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## Abstract

*Background Data and Objective:* There is anecdotal evidence that low-level laser therapy (LLLT) may affect the development of muscular fatigue, minor muscle damage, and recovery after heavy exercises. Although manufacturers claim that cluster probes (LEDT) maybe more effective than single-diode lasers in clinical settings, there is a lack of head-to-head comparisons in controlled trials. This study was designed to compare the effect of single-diode LLLT and cluster LEDT before heavy exercise. Materials and Methods: This was a randomized, placebo-controlled, double-blind cross-over study. Young male volleyball players (n=8) were enrolled and asked to perform three Wingate cycle tests after 4×30 sec LLLT or LEDT pretreatment of the rectus femoris muscle with either (1) an active LEDT cluster-probe (660/850 nm, 10/30 mW), (2) a placebo cluster-probe with no output, and (3) a single-diode 810-nm 200-mW laser. Results: The active LEDT group had significantly decreased post-exercise creatine kinase (CK) levels ( $-18.88 \pm 41.48$  U/L), compared to the placebo cluster group  $(26.88 \pm 15.18 \text{ U/L})$  (p < 0.05) and the active single-diode laser group  $(43.38 \pm 32.90 \text{ U/L})$  (p < 0.01). None of the pre-exercise LLLT or LEDT protocols enhanced performance on the Wingate tests or reduced post-exercise blood lactate levels. However, a non-significant tendency toward lower post-exercise blood lactate levels in the treated groups should be explored further. Conclusion: In this experimental set-up, only the active LEDT probe decreased post-exercise CK levels after the Wingate cycle test. Neither performance nor blood lactate levels were significantly affected by this protocol of pre-exercise LEDT or LLLT.

## Introduction

**T** IS COMPLEX AND DIFFICULT to evaluate muscle recovery and muscle damage in humans after high-intensity exercise. Direct analyses are only possible through muscle biopsy or magnetic resonance imaging, both of which are high-cost and have questionable accuracy.<sup>1</sup> The development of indirect analyses of physiological and biochemical markers have opened up new possibilities and they can be routinely performed in the laboratory.

Activity-related changes in plasma creatine kinase (CK) levels may be monitored not only as a marker to prevent overtraining, but as a way to identify an emerging state of muscle damage.<sup>2</sup> The CK level depends on the age, gender, ethnicity, muscle mass, and physical activity level of the athlete.<sup>3</sup> CK levels increase after exercise,<sup>4</sup> and serum CK

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levels vary with training level and the protocol used.<sup>5</sup> While athletes have higher CK levels at rest than non-trained individuals,<sup>6</sup> post-exercise increases are lower in athletes.<sup>7</sup> Individual muscle properties may also have an influence on variations in CK levels.<sup>8</sup>

The concentration of blood lactate, a glycolytic muscle metabolism by-product, is an excellent tool for sports training supervision,<sup>9</sup> and a valuable parameter related to the development of muscle fatigue. In a review of metabolic acidosis,<sup>10</sup> it was emphasized that the relationship between the lactic acid increase and the pH decrease should not be interpreted as a cause-and-effect link. Lactate accumulation is a good indirect indicator of increases in H<sup>+</sup> protons and decreases in blood and cellular pH. These factors promote metabolic acidosis, and consequently the development of skeletal muscle fatigue. Although some authors<sup>10–12</sup> have questioned the validity of using lactate concentrations as a parameter to determine muscle recovery after exercise, they continue to be widely used for this purpose.<sup>13–15</sup>

Recent studies of low-level laser therapy (LLLT) and the development of skeletal muscle fatigue have suggested its possible effects on CK activity and blood lactate production after exercise. After electrically-induced tetanic muscle contractions, increased CK levels and possible minor muscle damage have been observed in rats. LLLT administered prior to exercise appears to alter both CK levels and the expected decline in muscle power.<sup>16</sup> The reasons for these effects remain uncertain, but other studies of smooth muscle injury suggest that LLLT inhibits the release of reactive oxygen species and prevents muscle ischemia. In a recent in-vitro study of rat muscle cells, specific LLLT doses significantly decreased the production of reactive oxygen species (agents related to oxidative stress) and restored mitochondrial dysfunction.<sup>17</sup> With the latter factor in mind, it seems reasonable to irradiate as large a portion of the muscle belly as possible. Multi-diode cluster probes have been available for two decades. They typically have several visible red LED diodes and only a few infrared laser diodes. LEDs are cheaper to manufacture and they have larger spot sizes than standard diode lasers. However, many of the past trials of cluster probes have yielded negative results.<sup>18,19</sup>

The objective of this study was to evaluate the effect of two different light therapy applications (a single-diode probe versus an LED multi-cluster probe) on CK enzyme activity and the production and removal speed of blood lactate after a high-intensity exercise protocol.

## Materials and Methods

The study was designed as a cross-over randomized double-blind placebo-controlled trial. It was approved by the ethics committee of the Vale do Paraíba University (protocol number H04/CEP/2008). All subjects signed a written declaration of informed consent. The volunteers were recruited among young male volleyball players (n=8) from Rio Grande do Sul State (Caxias do Sul, Brazil).

## Randomization

All participants were subject to LLLT procedures and a performance test three times. The allocation to the LLLT procedure was subject to randomization in which simple drawing of lots (A, B, or C) determined whether the participants would receive active probe LLLT, active cluster LEDT, or placebo cluster LEDT. For participants drawing lot A, active probe LLLT was given at the first session, active cluster LEDT was given at the second session, and placebo cluster LEDT was given at the third session. For participants drawing lot B, active cluster LEDT was given at the first session, placebo cluster LEDT was given at second session, and active probe LLLT at the third session. For participants drawing lot C, placebo cluster LEDT was given at first session, active probe LLLT was given at the second session, and active probe LLLT was given at the second session, and active cluster LEDT was given at the second session, and active cluster LEDT was given at the second session, and active cluster LEDT was given at the second session, and active cluster LEDT was given at the third session.

All participants were crossed-over during the experiment in order to compare the outcomes after active probe LLLT, active cluster LEDT, and placebo cluster LEDT for each participant. The group allocation code from the drawing of lots was delivered to a technician who preset the laser control unit to either active or placebo mode. The allocation of treatments was concealed from participants, therapists, and observers.

#### Inclusion criteria

Inclusion criteria included: male volleyball players, who had been playing volleyball for at least 5 y, who were 17–20 y of age.



FIG. 1. Timeline of the study.

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## Exclusion criteria

Exclusion criteria included: any previous musculoskeletal injury to the hip, knee, or ankle; participation in less than 80% of the regularly scheduled physical training and volleyball sessions for the volleyball team; and players using any kind of nutritional supplements or pharmacological agents.

#### Procedures

**Period of evaluation**. Care was taken in obtaining standardization in the execution of the exercise protocols. Exercises were performed in a standard sitting position at approximately the same time of the day (to control for circadian rhythm). The performance and evaluation of the exercises were performed at three sessions (day 1, day 8, and day 15) on the same day of the week (Monday) at the same time of day (between 6:30 and 9:30 PM). No strenuous physical activity was permitted on the weekend before testing. The timeline of the experiment is shown in Fig. 1.

Fatigue test protocol. At the first (day 1), second (day 8), and third sessions (day 15) of the study, basal blood measurements (creatine kinase and lactate concentrations) were obtained from each subject. Immediately after this, all eight subjects were subjected to a standardized series of active muscle stretching exercises performed involving the plantar flexors, knee flexors, and the knee extensors (one round of 60 sec for each muscle group). Then each subject was seated on an ergometer cycle with the feet fixed to the pedals. The fatigue protocol consisted of a Wingate test. The Wingate test is defined as cycling at maximal speed for 30 sec with a load of 7.5% of the athlete's body weight. The researcher supervising the Wingate tests encouraged the athletes verbally throughout the 30-sec test period. A CEFISE® ergometer cycle model Biotec 2100 was used in all tests, and the load ranged from 5-7 kg according to body weight.

Light therapy and blinding procedures. At all sessions (day 1, day 8, and day 15), the participants either received a single treatment with an active probe laser (a single laser diode of 810 nm; THOR® Laser, London, U.K.), an active cluster LEDT laser, or a placebo cluster LEDT (both with a cluster with 34 LEDs of 660 nm and 35 LEDs of 850 nm; THOR® Laser), according to the results of the randomization procedure. The technician preset the laser unit according to the results of the randomization procedure, and he was instructed not to communicate the type of treatment given to the therapist, the participants, or the observers. Blinding was maintained by the use of opaque goggles by the participants and the therapist during light therapy. Active probe LLLT, active cluster LEDT, or placebo cluster LEDT were administered immediately after the stretching regimen, but immediately before the exercise fatigue test. Two irradiation points along the ventral side of the rectus femoris muscle belly were selected bilaterally (Fig. 2).

The irradiation was performed in contact mode with the laser probe or cluster held stationary with slight pressure at a 90° angle to the skin at each of the four treatment points. The laser parameters for active probe LLLT are summarized in Table 1, and the laser parameters for cluster LEDT (active and placebo) in Table 2.



FIG. 2. Irradiation points (black circles) used for active probe LLLT, active cluster LEDT, and placebo cluster LEDT.

After active or placebo light therapy had been administered, the participants were immediately repositioned and they begun the fatigue exercise protocol within 3 min.

#### Blood samples for creatine kinase concentration

Muscle damage was indirectly measured in the volleyball players with their levels of creatine kinase (CK). In order to

TABLE 1. LASER PARAMETERS FOR PROBE LLLT

Number of diodes: 1 laser diode Wavelength: 810 nm (infrared) Laser mode: Continuous output Optical output: 200 mW Spot size: 0.0364 cm<sup>2</sup> Power density: 5.50 W/cm<sup>2</sup> Energy: 6J at each point Energy density: 164.84 J/cm<sup>2</sup> at each point Treatment time: 30 sec at each point Number of irradiation points per muscle: 2 Total energy delivered per muscle: 12 J Application mode: Probe held stationary in skin contact at a 90° angle to the skin with slight pressure

TABLE 2. LASER FARAMETERS FOR CLUSTER LED	Table 2.	LASER PARAM	IETERS FOR	Cluster	LEDT
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Number of LEDs: 69 (34 red diodes and 35 infrared
Wavelength: 660 nm (red diodes) and 850 nm
LED mode: Continuous output
Optical output: 10 mW (red) and 30 mW
(for both red and infrared)
LED spot size: 0.2 cm <sup>2</sup> (for both red and infrared probes)
Power density: $0.05 \text{ W/cm}^2$ (for red) and $0.15 \text{ W/cm}^2$
(for infrared)
Energy: 41.7 J at each point (0.3 J from each red LED
and 0.9 J from each infrared LED)
Energy density: 1.5 J/cm <sup>2</sup> at each point (for red)
and $4.5 \text{J/cm}^2$ at each point (for infrared)
Treatment time: 30 sec at each point
Number of irradiation points per muscle: 2
Total energy delivered per muscle: 83.4 J
Application mode: Probe held stationary in skin contact at
a $90^{\circ}$ angle with slight pressure

measure the blood CK level, following aseptic cleaning of the ventral side of the dominant arm, a qualified nurse (blinded to treatment allocation) took one sample before stretching and treatment, and another blood sample 3 min after the exercises were completed. The blood analysis was performed with infrared spectrophotometry by an observer who was blinded to treatment allocation.

# Blood samples for measurement of lactate concentration

After aseptic cleaning of the index finger on the dominant side, a qualified nurse (blinded to group allocation) took one sample before the stretching procedure, and three other blood samples were taken at 3, 10, and 15 min after the exercises were completed. Lancets were used for obtaining the blood, and the samples were immediately analyzed with a portable lactate analyzer by an observer blinded to treatment allocation.

## Statistical analysis

Group means and standard deviations were calculated. An ANOVA test with Tukey-Kramer post-testing was used to test if there was a significant difference between the changes seen in the active probe LLLT group, active cluster LLLT group, and placebo cluster LLLT group. The significance level was set at p < 0.05.

## Results

Eight healthy young male volleyball players met the inclusion criteria and agreed to participate. Their average age

was 18.50 y ( $\pm$ 0.93 y), body weight was a mean of 81.65 kg ( $\pm$ 3.07 kg), and height was 189.25 cm ( $\pm$ 11.06 cm).

There were no significant differences between the pretreatment group levels of CK or blood lactate (Table 3).

The Wingate test revealed non-significant differences in the athletes' performance after the LLLT or LEDT procedures. Differences in peak power output, as well as mean power output, between the active probe LLLT group (12.20 W/kg  $\pm$  0.46; 9.55 W/kg  $\pm$  0.35), active cluster LEDT group (12.31 W/kg  $\pm$  0.83; 9.58 W/kg  $\pm$  0.57), and placebo cluster LEDT group (12.36 W/kg  $\pm$  0.59; 9.64 W/kg  $\pm$  0.39) were non-significant (p > 0.05), and F values were 0.2656 for peak power output and 0.3309 for mean power output. The results are summarized in Fig. 3.

All groups increased their blood lactate levels significantly from the baseline assessments to the post-exercise assessments. There were, however, no significant differences between the three treatment groups in the change in blood lactate levels at 3 min post-exercise (active probe LLLT 8.40 mmol/L $\pm$ 1.75; active cluster LEDT 8.78 mmol/L $\pm$ 1.74; and placebo cluster LEDT 8.38 mmol/L $\pm$ 2.59), at 10 min post-exercise (active probe LLLT 8.81 mmol/L $\pm$ 2.67; active cluster LEDT 9.29 mmol/L $\pm$ 2.94; and placebo cluster LEDT 10.29 mmol/L $\pm$ 1.89), and at 15 min post-exercise (active probe LLLT 8.93 mmol/L $\pm$ 2.22; active cluster LEDT 8.60 mmol/L $\pm$ 2.05; and placebo cluster LEDT 9.38 mmol/L $\pm$ 2.05; the F value was 1.128. The post-exercise results are summarized in Fig. 4.

The creatine kinase post-exercise levels showed that the active cluster group had decreased CK levels ( $-18.88 \text{ U/L} \pm 41.48$ ), compared to the increases seen in both the placebo cluster group ( $26.88 \text{ U/L} \pm 15.18$ ) (p < 0.05), and in the active probe group ( $43.38 \text{ U/L} \pm 32.90$ ). This difference was statistically significant (p < 0.01), but the difference in increases between the active probe group and the placebo cluster group was not statistically significant (p > 0.05), and the F value was 7.181. The results are summarized in Fig. 5.

## Discussion

In this study we evaluated the effects of light therapy on a test of athletic performance, minor post-exercise muscle damage, and exercise-induced changes in blood lactate levels and the subsequent removal of blood lactate. It appears that the pre-exercise light therapy protocols used in this study did not enhance the athletic performance of the athletes. The work performed during the Wingate test varied with <1%, which may indicate that we succeeded in standardizing the test procedure across the different intervention groups. The discrepancy between the positive results of the previous animal study and those of this trial can be attributed to different demands placed upon muscle performance by the Wingate test and by the tetanic contraction model used in the animal study. While the animal model only measured muscle performance performance performance by the study.

TABLE 3. BASELINE PRE-TREATMENT CK AND LACTATE LEVELS

Baseline pre-treatment levels	Active cluster probe	Active probe	Placebo cluster	Statistical difference
CK levels	190.75 U/L (±93.19)	232.13 U/L (±153.28)	192.50 U/L (±69.80)	Not significant
Blood lactate levels	1.55 mmol/L (±0.54)	1.54 mmol/L (±0.38)	1.66 mmol/L (±0.42)	Not significant

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**FIG. 3.** Peak power output and mean power output of the volleyball athletes during the Wingate test.

formance in a single muscle, the Wingate test involves muscle work performed by a number of different muscle groups, and our light therapy protocol only included irradiation of one of the involved muscle groups.

The non-significant findings for systemic changes in blood lactate levels were not surprising. They may be attributed to the experimental protocol used and the limitations in the number of irradiated points, and do not infer that light therapy is incapable of reducing the production of lactate. More studies are needed in which larger portions of the working muscles are irradiated by light therapy before the question of light therapy effects on blood lactate production can be adequately answered. Still, our results indicate that there appeared to be a tendency toward lower post-exercise blood lactate levels at 10 and 15 min, for both active treatment groups. Our study was probably too underpowered to detect if light therapy causes more rapid removal of lactate.



**FIG. 4.** Changes in blood lactate levels at the three time points post-exercise.



FIG. 5. Changes in creatine kinase levels post-exercise.

More studies of light therapy irradiation in which more of the working muscles are covered, and larger patient samples are needed to determine if light therapy causes more rapid removal of blood lactate after the Wingate test.

When it comes to precursors of muscle damage, our results of significantly decreased CK levels after active cluster LEDT are in accordance with those of a previous study, in which the authors found that cluster probe LLLT after heavy eccentric exercise reduced pain due to delayed-onset muscle soreness.<sup>20</sup> Increases in CK activity after exercise are an early marker of muscle injury, and have been used to avoid overtraining.<sup>2</sup> The preventive effects of LEDT on the inflammatory reaction to strenuous exercise could be valuable, and LEDT may then be used to make the make post-exercise recovery phase shorter. The observed decrease in CK levels could be related to the ability of LLLT to prevent muscle ischemia by reducing the release of reactive oxygen species and creatine phosphokinase activity, while levels of antioxidants and heat-shock proteins increase.<sup>21–22</sup> In a recent study,<sup>17</sup> it was found that LLLT improved mitochondrial function in muscle cells at doses of  $0.33-8.22 \text{ J/cm}^2$ , and that LLLT doses of  $0.33 \text{ and } 1.338 \text{ J/cm}^2$ reversed the dysfunctional state induced by electrical stimulation. This effect might possibly have contributed to the decreases seen in CK levels in our study.

Other possible explanations for the difference in CK levels seen between the active treatment groups include differences in the size of the irradiated areas and in the laser parameters used. Our selection of treatment probes was governed by their commercial availability. In addition, we wanted to test if the marketing claims of cluster probe manufacturers were true, namely that cluster probes yield better clinical results with the same irradiation times. In the case of CK levels, their statement seems to be true, but whether this advantage is caused by a larger versus a smaller irradiated area, the type of LED laser probe used, the lower output and power density (10 and 30 mW versus 200 mW), the different wavelengths (660 and 850 mW versus 810 nm), or the use of two wavelengths versus one wavelength, remain unanswered.

One should be careful in applying the results reported here more broadly, because of the small sample size (n = 8), although the sample was quite homogeneous. However, the largest problem with the small sample size is that it is more prone to type II errors, in which the true differences between the interventions are not detected because of the low statistical power.<sup>23</sup> The lack of influence on post-exercise lactate levels seen in the laser groups may be the result of low statistical power, rather than a true lack of effect, as both laser groups showed lower values at both 10 and 15 min post-exercise. This question needs further investigation in larger samples before any conclusions can be drawn. Still, in our experiment we detected a significant reduction in CK levels in the cluster group compared to placebo. It is more likely that this represents a true difference, as LLLT previously has been shown to reduce inflammation in skeletal muscle,<sup>20</sup> and to protect against ischemic muscle damage.<sup>21</sup> Also, as the cluster probe irradiated a larger portion of the muscle tissue, it would be logical to assume that the cluster group would yield the best results. As such, this small trial of ours represents a useful approach for future avenues for light therapy research, and the clinical significance of our findings needs to be validated in a setting in which both physical performance outcomes and post-exercise recovery are taken into account.

## Conclusion

We conclude that in this experimental protocol with 120 sec of pre-exercise LEDT administered at four points over the rectus femoris muscle, only the dual-wavelength (660 and 850 nm) cluster LED probe decreased post-exercise CK levels after Wingate testing. Performance was not enhanced by pre-exercise LLLT or LEDT, and blood lactate levels were not significantly affected by LLLT or LEDT. These findings may indicate that pre-exercise LLLT may protect the muscle against minor damage and the inflammatory reaction seen after strenuous exercise.

Because of the large differences in irradiated areas by single diode LLLT (0.14 cm<sup>2</sup>), and narrow-band cluster multidiode LEDT (55.2 cm<sup>2</sup>), the results do not infer that LEDT is more effective than LLLT. On the contrary, several trials have found that laser light sources with narrower bandwidth and higher coherence achieve significantly superior results over LEDT with equal doses in both inflammation and wound healing experimental models. Still, LEDT have some advantages, such as larger spot size and lower cost, which may outweight LEDTs inferior effect when large areas need to be irradiated. Further research is necessary to determine the optimal laser or LED parameters for this application, and to investigate whether lactate removal can be accelerated by pre-exercise LLLT or LEDT.

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## **Disclosure Statement**

No conflicting financial interests exist.

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