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Effect of Cluster Multi-Diode Light Emitting Diode Therapy (LEDT) on Exercise-Induced Skeletal Muscle Fatigue and Skeletal Muscle Recovery in Humans

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Background and Objectives: There are some indications that low-level laser therapy (LLLT) may delay the development of skeletal muscle fatigue during high-intensity exercise. There have also been claims that LED cluster probes may be effective for this application however there are differences between LED and laser sources like spot size, spectral width, power output, etc. In this study we wanted to test if light emitting diode therapy (LEDT) can alter muscle performance, fatigue development and biochemical markers for skeletal muscle recovery in an experimental model of biceps humeri muscle contractions.

Study Design/Materials and Methods: Ten male professional volleyball players (23.6 [SD \pm 5.6] years old) entered a randomized double-blinded placebo-controlled crossover trial. Active cluster LEDT (69 LEDs with wavelengths 660/850 nm, 10/30 mW, 30 seconds total irradiation time, 41.7 J of total energy irradiated) or an identical placebo LEDT was delivered under double-blinded conditions to the middle of biceps humeri muscle immediately before exercise. All subjects performed voluntary biceps humeri contractions with a workload of 75% of their maximal voluntary contraction force (MVC) until exhaustion.

Results: Active LEDT increased the number of biceps humeri contractions by 12.9% (38.60 [SD \pm 9.03] vs. 34.20 [SD \pm 8.68], $P=0.021$) and extended the elapsed time to perform contractions by 11.6% ($P=0.036$) versus placebo. In addition, post-exercise levels of biochemical markers decreased significantly with active LEDT: Blood Lactate ($P=0.042$), Creatine Kinase ($P=0.035$), and C-Reactive Protein levels ($P=0.030$), when compared to placebo LEDT.

Conclusion: We conclude that this particular procedure and dose of LEDT immediately before exhaustive biceps humeri contractions, causes a slight delay in the development of skeletal muscle fatigue, decreases post-exercise

blood lactate levels and inhibits the release of Creatine Kinase and C-Reactive Protein. *Lasers Surg. Med.* 41:572–577, 2009. © 2009 Wiley-Liss, Inc.

Key words: light emitting diode therapy; skeletal muscle performance; skeletal muscle damage; high-intensity exercise

INTRODUCTION

The development of skeletal muscle fatigue is characterized by a time-dependent decrease in muscle strength [1]. Several factors such as the types and intensity of exercise, the muscle groups involved, and the local physical and biochemical environment affect fatigue development, which is a complex and multifaceted process involving physiological, biomechanical, and psychological elements [2]. Age and sex also determine ability to contract skeletal muscle and to withstand fatigue development [3,4].

During high intensity exercise in anaerobic conditions, oxidative stress occurs and reactive oxygen species (ROS)—signaling agents are being released. Amongst other things, ROS inhibit mitochondrial function, which in turn is known to cause muscle cell depolarization and reduced force [5].

Muscle damage can occur together with skeletal muscle fatigue after strenuous sporting activities [6]. Recent studies of low level laser therapy (LLLT) administered

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before the development of skeletal muscle fatigue during exercise, have suggested possible effects on Creatine Kinase (CK) enzyme activity and blood lactate production. In an animal study with electrically induced tetanic muscle contractions in rats, increased CK levels were observed, indicating possible minor muscle damage. These factors were altered when LLLT was administered prior to the exercise [7]. Other animal studies in smooth muscle injury, suggest that LLLT inhibits the release of ROS and prevents muscle ischemia [8]. Whether these positive results can translate into positive effects on human athletes has only been sparsely studied in randomized controlled studies. There is also a large variation of different treatment protocols and commercially available therapeutic laser/light devices on the market, and the trial results are mixed. In one trial with an 808 nm laser scanning device, doses of 3 and 7 J over quadriceps muscle, the authors found no significant effect over control for muscle force levels during repeated knee extensions [9].

Our research group has previously evaluated the effects of LLLT administered to four small points of the biceps muscle belly with single diode lasers of red [10] and infrared [11] wavelengths on skeletal muscle fatigue. If the intention is to cover a large part of the exercised muscle, then multi-diode cluster probes with several visible red LED diodes or infrared laser diodes may be more suitable for this purpose. LEDs are cheaper to manufacture and they have larger spot sizes than laser diodes. However, results from available trials with cluster probes in delayed muscle soreness are inconsistent and often with non-significant results [12,13]. We are not aware of any studies investigating LEDT effects on muscle performance, and there is a lack of data on possible LEDT effects on biochemical markers of muscle damage and recovery.

The objective of this study is to evaluate effects of multi-diode cluster LEDT in skeletal muscle fatigue development, muscle damage and recovery after exercise. We used a randomized placebo-controlled crossover design, with the number of repetitions and time to fatigue as an outcome of fatigue development. In addition we measured CK enzyme activity and C-Reactive Protein (CRP) levels as markers of early muscle damage, while changes in blood lactate levels were taken as an indirect measure of post-exercise recovery.

METHODS

The study was designed as a randomized double-blinded placebo-controlled cross-over 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 professional male volleyball players ($n = 10$) in the same team of the highest national sporting level in Brazil.

Randomization and Blinding Procedures

Randomization was performed by a simple drawing of lots (A or B), which determined whether active LEDT (A) or

placebo LEDT (B) should be given at the first exercise session. At the second session, participants were crossed over to receive whichever treatment A or B was not given at the first session. The code from the drawing of lots was delivered to a technician who preset the control unit accordingly to either an active LEDT or placebo LEDT mode. The technician was also instructed not to communicate the type of treatment given to either the participants, the therapist applying the LED source to the biceps, or the observers. Thus, the allocation to treatments was concealed to participants, therapist and observers. Blinding was further maintained by the use of opaque goggles by participants and the therapist during LEDT procedures.

Inclusion Criteria

The following inclusion criteria were used:

Healthy male volleyball players aged between 18 and 36 years, who had played volleyball at a professional level for at least 2 years.

Exclusion Criteria

The following exclusion criteria were used:

Any previous musculoskeletal injury to the shoulder or elbow regions, participation in less than 80% of the scheduled team physical training and volleyball sessions for the last 3 months, and players using any kind of nutritional supplements or pharmacological agents.

Procedures

In order to provide a stable 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 with a two-decimal scale was used.

Maximum voluntary contraction (MVC) test. Athletes were familiarized to elbow flexion-extension exercises with an adaptation period of 2 weeks. After 2 weeks of familiarization with the exercise, we performed a maximal contraction force test that consisted of one single repetition of flexion to 90° from full extension of the elbow in order to evaluate the strength of the biceps muscle while the subject was seated in the Scott bench. Free weights ("halters") were used, and the specific individual weight (load) corresponding to 75% of MVC, was established for each subject.

Period of evaluation. Care was taken in obtaining standardization in the execution of the exercise protocols. Exercises were performed in a standardized sitting position, and the exercise tests were performed in two sessions (day 1 and day 8) at the same day of the week (Monday) in the same period of the day (between 8:30 and 11:30 AM). Any hard physical activity was not allowed in the weekend before testing.

Fatigue protocol. At the first exercise test session (day 1) and second exercise test session (day 8) of the study, basal blood measurements were obtained for each subject from the ventral side of dominant arm. Immediately after this, all 10 subjects were submitted to a series of muscle

stretching exercises involving all the major muscles of the upper extremities (two rounds of 60 seconds for each muscle group) and finishing with the flexor muscles of the elbow. Then, each subject was seated in the Scott bench, with the knee and the hip flexed at 90°. Using a free weight halter, the previously defined personal weight load (=75% of maximal load) was performed for each subject. A goniometer was fixed to the Scott Chair to measure the flexion angle and controlled by an observer. The number of repetitions in the exercise fatigue test was counted by the same observer, and the total time to finish the effort was measured by a second observer.

LEDT procedure. At two sessions (day 1 and day 8), the participants either received a single treatment of active cluster LEDT or placebo cluster LEDT (both using a cluster with 34 LED diodes of 660 nm and 35 LED diodes of 850 nm manufactured by THOR[®] Photomedicine, London, UK) according to the result of the randomization procedure. Active cluster LEDT or placebo cluster LEDT was administered immediately after the stretching regimen, but immediately before the exercise fatigue test (exactly 180 seconds before). One irradiation point exactly in the middle of the ventral side of the biceps muscle belly was selected.

The irradiation was performed in contact mode with the LED cluster held stationary with slight pressure at a 90° angle to the skin in the treatment point for 30 seconds. Parameters for the cluster LEDT (active and placebo) are summarized in Table 1.

After active LEDT or placebo LEDT had been administered, participants were immediately repositioned and started the fatigue exercise protocol within an exact interval of 180 seconds.

Blood Samples for Blood Lactate, Creatine Kinase, and C-Reactive Protein Analysis

Blood samples were taken, lactate levels were measured as well as CK activity and CRP levels for muscle damage

and the inflammation. In order to measure those parameters, a qualified nurse (blinded to group allocation) performed aseptic cleaning of the ventral side of the dominant arm, and took one sample before stretching and treatments, and another blood sample exactly 3 minutes after the exercises were completed. The samples were frozen and after 1 week of the end of each phase the blood analysis was performed with infrared spectrophotometry, using a spectrophotometer (FEMTO[®], Brazil) and specific kits for analysis of Blood Lactate (Bioclin[®], Brazil) and CK (Labtest[®], Brazil). The analysis of CRP was performed by the agglutination method using a specific analysis kit (Wiener Laboratorios[®], Argentina). All blood analyses were performed by an observer who was blinded to treatment allocations.

Statistical Analysis

Group means and their respective standard deviations were used for statistical analysis. A two-sided paired *t*-test was used to test if there was a significant difference in change between active cluster LEDT or placebo cluster LEDT treatments. The significance level was set at $P < 0.05$.

RESULTS

Ten healthy male professional volleyball players met the inclusion criteria and gave their signed informed consent for participation. Their average age was 23.6 years old (SD ± 5.6), and their body weight was a mean of 87.5 kg (SD ± 9.1) and their body height was 194.5 cm (SD ± 6.6).

The mean number of repetitions performed in the exercise test was 12.9% higher (38.6 [SD ± 9.03]) when the volunteers received active LEDT versus placebo LEDT (34.20 [SD ± 8.6], $P = 0.021$). The results are summarized in Figure 1.

The results for elapsed time in the exercise tests, was 11.6% higher after active LEDT (47.37 seconds [SD ± 11.50]) than after placebo LEDT (42.46 seconds [SD ± 13.81]), and this difference reached statistical significance ($P = 0.036$). The results for elapsed time are summarized in Figure 2.

The blood lactate levels before exercise tests were not significantly different at 3.40 mmol L⁻¹ (SD ± 1.07) for active LEDT and 3.70 mmol L⁻¹ (SD ± 1.25) for placebo LEDT ($P > 0.05$). Blood lactate levels increased during exercise in all groups. However, there was significant difference in the change of blood lactate levels from pre-exercise to post-exercise measurements ($P = 0.042$) between active LEDT at 8.20 mmol L⁻¹ (SD ± 3.99) and placebo LEDT at 11.50 mmol L⁻¹ (SD ± 3.21). The results are summarized in Figure 3.

The CK activity before the exercise tests were 53.62 U L⁻¹ (SD ± 23.37) for active LEDT and 52.91 U L⁻¹ (SD ± 40.78) for placebo LEDT ($P > 0.05$). The change in CK activity before and after exercises was significantly lower ($P = 0.035$) after active LEDT (-3.04 U L⁻¹ [SD ± 4.47]) than after placebo LEDT (4.33 U L⁻¹ [SD ± 8.65]). The changes in CK activity are summarized in Figure 4.

TABLE 1. Parameters for Cluster LEDT

| |
|--|
| Number of LEDs: 69 (34 red LEDs and 35 infrared LEDs) |
| Wavelength: 660 nm (red) and 850 nm (infrared) |
| Frequency: Continuous output |
| Optical output: 10 mW (red) and 30 mW (infrared) |
| LED spot size: 0.2 cm ² (for both—red and infrared), total spot sizes 13.8 cm ² |
| Power density: 0.05 W cm ⁻² (for red) and 0.15 W cm ⁻² (for infrared) |
| Energy: 41.7 J (0.3 J from each red LED 0.9 J from each infrared LED) |
| Energy density: 1.5 J cm ⁻² (for red) and 4.5 J cm ⁻² (for infrared) |
| Treatment time: 30 seconds |
| Number of irradiation points per muscle: 1 |
| Total energy delivered per muscle: 41.7 J |
| Total area irradiated: 13.8 cm ² |
| Application mode: Cluster held stationary in skin contact with a 90° angle and slight pressure |

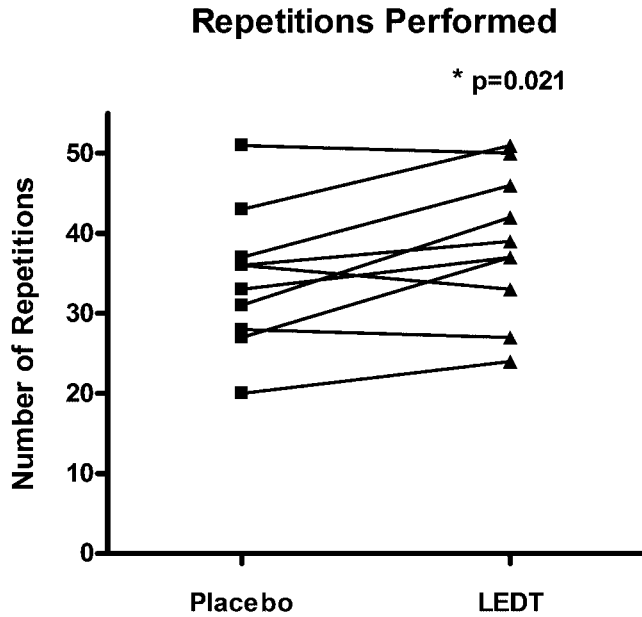


Fig. 1. Repetitions performed by volleyball athletes. The number of repetitions ($\approx 75\%$ MVC) performed before exhaustion for each participant after placebo LEDT and active LEDT.

The levels of CRP before exercise tests were $1536.00 \text{ mg dl}^{-1}$ ($\text{SD} \pm 742.09$) for active LEDT and $1077.60 \text{ mg dl}^{-1}$ ($\text{SD} \pm 643.24$) for placebo LEDT ($P > 0.05$). After exercises, the change in CRP level ($-364.80 \text{ mg dl}^{-1}$ [$\text{SD} \pm 616.86$]) was significantly higher ($P = 0.030$) after active LEDT than after placebo LEDT (28.80 mg dl^{-1} [$\text{SD} \pm 361.65$]). The changes in CRP levels are summarized in Figure 5.

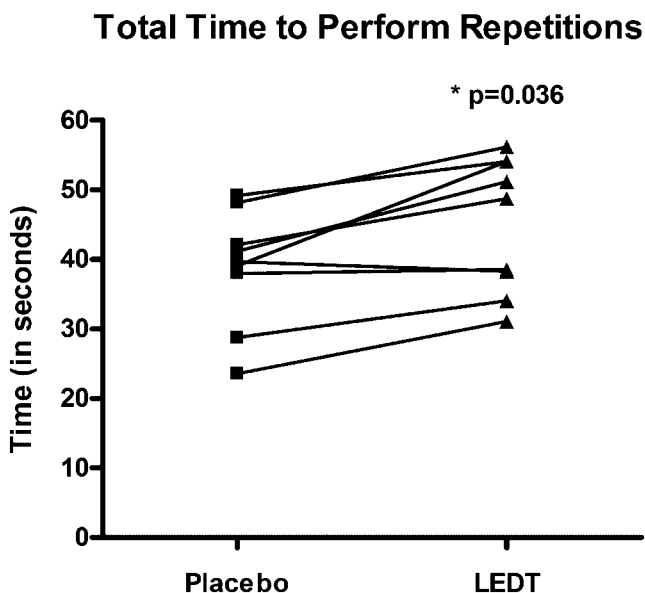


Fig. 2. Time used to perform repetitions. Time elapsed before exhaustion for each participant after placebo LEDT and active LEDT.

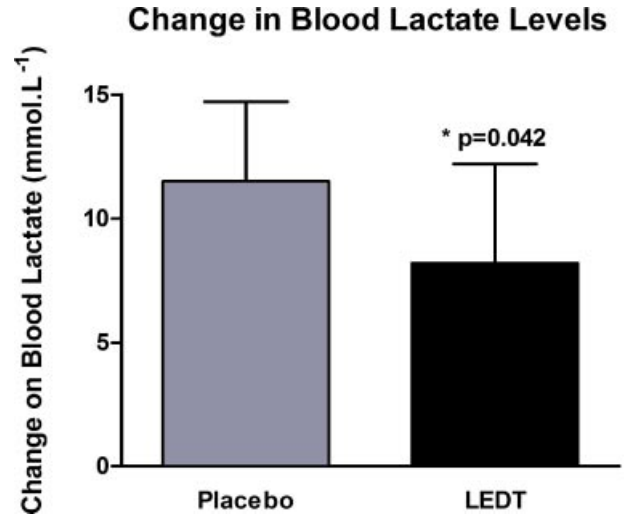


Fig. 3. Blood Lactate changes after exercise. Active LEDT (black bar) induced a significantly lower increase in the blood lactate level than placebo LEDT (gray bar). Error bars indicate SDs of change.

DISCUSSION

In the current study we found that irradiation with this particular LEDT protocol, significantly increased the number of performed muscle contractions with 75% of their respective MVC. Seven out of 10 participating athletes had a higher number of repetitions, while eight out of 10 athletes had a longer time to exhaustion in favor of active LEDT over placebo LEDT. To our knowledge, this is the first study which shows improved muscle performance after LEDT irradiation. In a previous study, we found no effect of LEDT administered to the quadriceps muscles before a Wingate cycling test [14], but this may be due to the

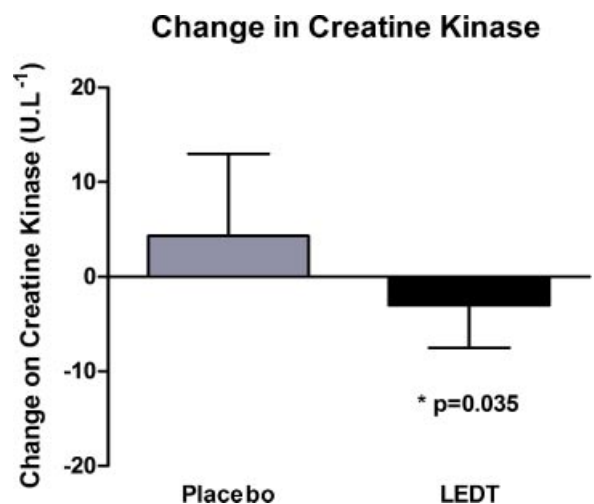


Fig. 4. Creatine Kinase (CK) changes after exercise. Active LEDT (black bar) resulted in a significantly lower increase in the CK activity than placebo LEDT (gray bar). Error bars indicate SDs of change.

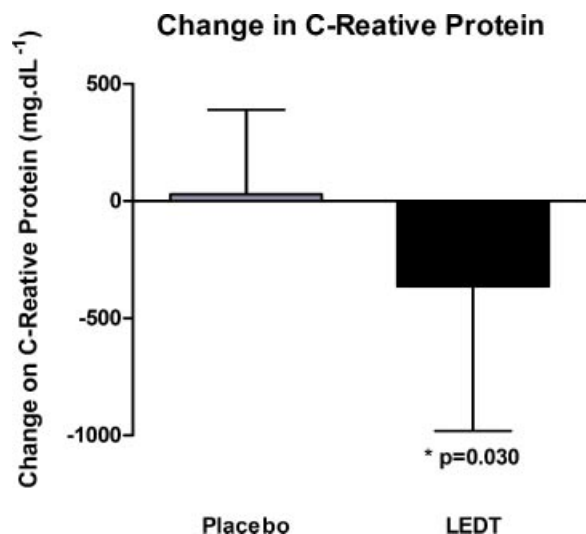


Fig. 5. C-Reactive Protein (CRP) changes after exercise. Active LEDT (black bar) resulted in a significantly smaller increase in the CRP level after exercise than placebo LEDT (gray bar). Error bars indicate SDs of change.

fact that an insufficient area of the involved muscles was irradiated. LLLT scanning devices may cover larger areas of muscles, but they have yet to prove their efficacy in controlled trials of skeletal muscle fatigue [9].

In the current study, we also observed a decrease in blood lactate levels at 3 minutes post-exercise after active LEDT was used. The decrease in blood lactate concentrations in the current study is different from the results of two of our LLLT studies [10,11]. In another recent study from our group [15] we found reduced blood lactate levels in the 30 seconds Wingate cycle test. However, blood lactate levels were only reduced at 15 minutes after cycling, whereas there were no significant differences at 5 and 10 minutes after cycling. The lower post-exercise blood lactate levels after active LEDT could be caused by several factors, and improved microcirculation is one possibility [16]. But it could also be caused by a reduction in skeletal muscle fatigue or improved lactate removal.

This study adds further support to hypothesis that post-exercise CK activity can be decreased by LLLT or LEDT. Similar findings on CK levels were observed after irradiation with an 830 nm single probe [15]. The decrease in CK activity could mean that LLLT can prevent muscle ischemia by reducing ROS release and creatine phosphokinase activity, while levels of antioxidants and heat shock proteins increase [17,18]. In a recent study by other authors [8], LLLT improved mitochondrial function in muscle cells at doses of 0.33–8.22 Jcm⁻² and LLLT doses of 0.33 and 1.338 Jcm⁻², and reversed the dysfunctional state induced by electrical stimulation. This mechanism could possibly contribute to the observed decrease in CK activity, and the increase in time before exhaustion in the current study.

This study also indicates that not only LLLT, but LEDT as well, can induce an anti-inflammatory response with a

significant reduction of CRP levels. It is well known that muscle damage often occurs after high intensity exercise, and is accompanied by an inflammatory response [19]. Previous studies have shown that inflammatory responses occur after high intensity exercise [19–22], and one study found a reduction in delayed onset muscle soreness after eccentric exercise [23]. It is surprising that CRP levels actually are significantly lower than the pre-exercise level for the LEDT group. We cannot offer any obvious explanation for this, except that LEDT seems to be a significant tool for controlling post-exercise inflammation.

There are several limitations to our study. We have used a pragmatic perspective to see if a commercially available LEDT cluster device could induce some of the same effects in muscle fatigue as we previously have observed with LLLT devices. It is not surprising to find that narrow-band LEDT induce some of the same effects as LLLT in fatigue development, but because of differences treatment parameters, it is not possible to determine which is most effective of the two therapies for preventing muscle fatigue and muscle damage. There is also too little data available for determining the optimal relationship between dose, power density, and irradiation time for LLLT and LEDT respectively in muscle fatigue development. In this small study we have observed a small increase (12.9%) in muscle performance over placebo, which was slightly less than what we observed in previous studies with 655 and 830 nm single diode LLLT [10,11]. But one should be careful in generalizing these results, because of the small sample size in these studies. More large scale LEDT studies are needed before certain conclusions can be drawn.

For LEDT, the reduction of blood lactate levels already at 3 minutes post-exercise only, may have been specific for the biceps contraction model. In more complex muscle work like the Wingate cycle test, it seems to take a little longer for the effect on blood lactate to emerge. But this needs to be verified or refuted in future studies. It must also be added that although the biochemical markers of post-exercise inflammation and muscle damage are reduced, more studies are needed to verify if these LEDT/LLLT effects can be translated into an enhancement of clinical recovery after high intensity exercise.

CONCLUSION

In this study we have shown that local irradiation of the biceps humeri muscle with this particular protocol of dual wavelength narrow-band LEDT, can delay the development of peripheral fatigue during exercise, decrease post-exercise blood lactate levels, and inhibit the release of inflammatory biomarkers. The LEDT effect on muscle performance was slightly smaller than in previous studies with LLLT. Whether this represents a true difference between the two therapies must be determined by more optimal dose-finding studies in the future.

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