Light Emitting Diode Therapy (LEDT) Applied Pre-Exercise Inhibits Lipid Peroxidation in Athletes After High-Intensity Exercise. A Preliminary Study

ABSTRACT

Oxidative stress is the term generally used to describe the damage caused by imbalance between pro-oxidants and antioxidants in the organism. The increase in the O2 consumption induced by physical exercise is associated with the increase of reactive oxygen species (ROS) being these species inducers of oxidative stress. Although the evidence indicates a probable inhibitory effect of the light emitting diode therapy (LEDT) on the production of ROS, there are no studies observing this effect in humans. This preliminary study has the aim to verify the effects of LEDT applied before high-intensity exercise on lipid peroxidation, measured through blood levels of ThioBarbituric Acid Reactive Substances (TBARS). Six male volleyball athletes were submitted to two situations: active LEDT and placebo LEDT. Performance in the exercise protocol showed no difference (p>0.05) between the two situations in peak power, average power and fatigue index. The results related to lipid peroxidation were: at active LEDT situation, it was not possible to observe statistically significant difference (p>0.05) between pre and post exercise levels (6.98 ± 0.81 and 7.02 ± 0.47 nmol/mL); at placebo LEDT situation, statistically significant difference (p=0.05) was observed between pre and post exercise levels (7.09 ± 1.28 and 8.43 ± 0.71 nmol/mL). These results show that active LEDT seems to be effective in controlling lipid peroxidation in athletes submitted to intense exercise.

Keywords: phototherapy, lipid peroxidation, sport

INTRODUCTION

Oxidative stress is the term generally used to describe the damage caused by imbalance between pro-oxidants and the antioxidant defense. Such fact may occur both by increase in the formation of reactive oxygen species (ROSs) and by decrease of capacity of cellular antioxidant defense. This damage includes increase of lipid peroxidation levels (oxidation of the lipid layer of the cell membrane), increase in the carbonylation of proteins and even damage in the intracellular DNA, which affects the intracellular metabolism and can cause apoptosis (cell death)(1-4).

Physical exercise promotes increase in the energetic demand of up to 35 times compared to rest(5). Knowing that about 2% to 5% of the O2 consumed gives origin to ROS in the mitochondria(2,6), the increase in O2 consumption induced by physical exercise is associated with the increase of the ROS being these inducers of oxidative stress. Dillard et al.(7) were the pioneers in demonstrating that physical exercise induces increase of lipid peroxidation. After 1982, when Davies et al.(8) demonstrated the oxidative damage caused by exhaustive exercise, considerable amount of experimental evidence has come out indicating thus a cause-effect correlation between oxidative stress and muscle fatigue(9). However, it has not been established yet whether lipid peroxidation is the cause or the consequence of tissue damage caused by exercise(11).

Some authors believe that oxidative stress can be harmful to sports performance(9,10), although there is still an evidence gap which supports such hypothesis. Studies performed with animals and isolate fibers indicate that increase of the ROSs induced by exogenous sources can harm muscle performance(11). Nevertheless, some authors suggest that the main effect of oxidative stress occurs in the long run, in the overreaching induction, an example of metabolic imbalance related to the initial status of overtraining(12,13). Although antioxidant supplementation has proved to be efficient in reducing oxidative stress induced by physical exercise in humans(14), there is no cohesive evidence which supports that sports performance improves as a response to lower level of oxidative stress(15).

In a recent study involving electrical stimulation in culture of muscle cells of rats, Xu et al.(16) have verified that the treatment with phototherapy significantly decreased the production of the ROSs...
and restored the mitochondrial function. The authors suggest that phototherapy is an original and non-invasive therapy in the treatment of muscle fatigue induced by exercise, tissue injury and other processes in which the mitochondrial function plays a key-role. Phototherapy has also demonstrated to be efficient in preventing oxidative stress after muscle injury induced by mechanical trauma in animal models\(^\text{17,18}\). In the pioneer study on phototherapy and muscle fatigue, Lopes-Martins et al.\(^\text{19}\) have observed the protective effect of phototherapy on the muscle damage in rats submitted to a protocol of muscle contractions generated by electrical stimulation. In humans, Leal Junior et al.\(^\text{20,21}\) have demonstrated that phototherapy using low level laser therapy (LLLT) can present positive effects in muscle fatigue attenuation as well as in post-exercise muscle recovery\(^\text{22}\) when the therapy is applied prior to the exercise performance. Moreover, Leal Junior et al.\(^\text{23,24}\) have also observed that phototherapy with light emitting diode therapy (LEDT) has effects similar to the LLLT concerning the attenuation of muscle fatigue and improvement of post-exercise recovery.

Although evidence indicates a probable inhibiting effect of phototherapy on the production of Ross, and consequently a protective effect against oxidative stress, until the present moment there are no studies which observe such possibility in athletes. Therefore, this preliminary study has the aim to verify the effect of LEDT concerning the attenuation of muscle fatigue and improvement of post-exercise recovery.

**METHODOLOGY**

A random clinical, placebo-controlled, double blind and crossed assay was performed. The study was approved by the Research Ethics Committee (REC) of the University of the Paraíba Valley and all participants signed the Free and Clarified Consent Form. The volunteers were recruited among young male volleyball athletes (\(n = 6\)) members of the youth team of the University of Caxias do Sul, having the following exclusion criteria been adopted: age below 17 years; time of practice below five years; any previous musculoskeletal injury in the hip, knee or ankle regions; participation below 80% of the team activities; payers using any kind of nutritional supplement or pharmacological agent.

All athletes went twice to the laboratory and were submitted to two situations: active LEDT application and placebo LEDT application. In both sessions, the following protocol was followed: initially, basal blood collection of the athlete was performed; afterwards, a standardized a muscle stretching protocol; subsequently, active or placebo LEDT application according to the previous randomization; three minutes after the end of the LEDT, application of the muscle fatigue inducing protocol (MFIP); three minutes after the end of MFIP; a new blood sample was collected, finalizing the protocol. Further details on the used procedures are presented in the following sessions.

**Randomization**

The LEDT application was defined through randomization in which the subjects were separated in two groups (A and B), which determined the order of application of the LEDT modalities. Concerning group A members, on the first session the active LEDT application was performed and on the second session, the placebo LEDT was applied. Concerning group B members, on the first session placebo LEDT was applied and active LEDT was applied on the second session. Randomization was full responsibility of a technician who also in charge of programming the LEDT equipment, where he determined the kinds of application (active or placebo) for each athlete, according to previous randomization. This technician also received instructions not to communicate the kind of treatment used to the researcher responsible for the LEDT application, to the volunteers and to the researchers responsible for the MFIP and for the blood collections and analyses.

**Assessment period**

The procedures were carried out exactly the same way on the two exercise sessions, which occurred with a one-week interval between them, on the same weekday (Monday), at the same time of the day (between 18:30h and 21:30h), and any high-intensity physical activity was prohibited during the weekends prior the tests. We can mention the exercises performance at approximately the same time the day (for control of the circadian rhythm), besides the recommendations to the athletes concerning the kind of suitable meals for the day of the procedures and interval between the last meal and the moment of the assessment are some careful measures taken for standardization in the exercise protocol performance.

**LEDT application protocol**

On both exercise sessions (tests), the participants received LEDT application (active or placebo) through an equipment brand name THOR® (London, United Kingdom) using a cluster with 34 LED diodes of 660nm and 35 LED diodes of 850nm, also manufactured by THOR® (London, United Kingdom), according to the randomization result. The active or placebo LEDT application was performed after a randomized stretching set and three minutes before the MFIP. Both active and placebo LEDT applications were administered by one of the researchers in isolate environment, and only the volunteer and the researcher were present. Eye protection (matte goggles) was used by the participants to avoid visualization or perception if they were receiving active or placebo LEDT application, since it does not cause any thermal sensation to the volunteer. The therapy was administered on the knee extensor musculature of the volunteers, the application points were selected the same way as in the study by Leal Junior et al.\(^\text{23}\), according to illustration in (figure 1).

Active LEDT application was performed by direct contact of the cluster with the skin, which was placed still with light pressure, at a 90 degree angle on each of the irradiation points. The active LEDT parameters are the same used by Leal Junior et al.\(^\text{29}\), and are summarized on table 1. The placebo LEDT application was performed exactly like the active one; however, with the equipment on placebo mode (without active irradiation).

**Muscle fatigue inducing protocol (MFIP)**

MFIP adopted (started three minutes after the end of the active or placebo LEDT application), was composed of a Wingate test performed on cycle ergometer with the athlete with feet attached to the pedals and having received instructions to pedal with maximum velocity during 30 seconds (with verbal stimulation during the entire test), using load 7.5% of his body mass. The
Blood samples

Antiseptic cleansing of the ventral region of the dominant arm was performed before each blood collection from which a qualified nursing professional collected 10ml of blood (using disposable gloves, syringes and needles). Each athlete was submitted to two collections in each session, one before the stretching and the LEDT application (active or placebo) and the other exactly three minutes after the end of the MFIP. The collected material was transferred to non-heparinized tubes and was subsequently centrifuged and frozen for further analysis of the level of ThioBarbituric Acid Reactive Substances (TBARS).

ThioBarbituric Acid Reactive Substances (TBARS)

As indication of lipid peroxidation, formation of ThioBarbituric Acid Reactive Substances (TBARS) was used during a heating reaction of the acid, which is widely adopted as a method fairly sensitive of measurement of oxidative stress.(25) In summary, the blood samples were mixed with the 10% trichloroacetic acid and to the 0.67% thiobarbituric acid, and then heated in boiling water bath for 15 minutes. The ThioBarbituric Acid Reactive Substances were determined by absorbance at 535nm. The results were expressed in nmol/ml.

Statistical Analysis

The means of the groups and their respective standard deviations were expressed in the results. A paired and two-tailed t test was used to verify whether there was statistically significant difference in the pre and post-exercise oxidative stress levels for the active and placebo LEDT application. The significance level adopted was of 5% (p < 0.05).

RESULTS

Six male healthy and young adult volleyball athletes who were within the inclusion criteria participated in this study. Mean age of the athletes was 18.57 years (± 0.98), mean body mass 82.03kg (± 3.11) and mean stature 188.71cm (± 11.83).

Performance of athletes in the Wingate test did not reveal statistically significant difference in the peak power or mean power between active LEDT (12.22W.kg⁻¹ ± 0.82; 9.54W.kg⁻¹ ± 0.60) and placebo LEDT (12.29W.kg⁻¹ ± 0.60; 9.65W.kg⁻¹ ± 0.42) (p > 0.05). Concerning the fatigue index (an indirect variable measured through minimal and maximal performance reached during the test) statistically significant differences have not been found either (p > 0.05) between active LEDT (39.64% ± 6.36) and placebo LEDT (39.88% ± 4.38).

The results related to oxidative stress, measured by the TBARS levels, are demonstrated in figure 2. In the active LEDT situation, statistically significant difference was not observed (p > 0.05) between the pre and post-exercise levels (6.98 ± 0.81 and 7.02 ± 0.47nmol/ml). Nevertheless, the placebo LEDT situation demonstrated statistically significant difference (p = 0.05) between the pre and post-exercise levels (7.09 ± 1.28 and 8.43 ± 0.71nmol/ml).

When the variation of the TBARS concentrations in the pre and post-exercise assessments was considered, statistically significant difference was observed (p = 0.02) between active LEDT (1.34 ± 1.29nmol/ml) and placebo LEDT (0.06 ± 0.53nmol/ml), according to what was illustrated in figure 3.

Figure 1. Point used in the LEDT application (active or placebo).

Figure 2. Comparison between the TBARS levels before (PRE) and after (POST) exercise at the active LEDT and placebo LEDT situations (*p = 0.05).

Table 1. Parameters for active LEDT.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of diodes</td>
<td>69 (34 red diodes and 35 infrared diodes)</td>
</tr>
<tr>
<td>Wave length</td>
<td>660nm (red diodes) and 850nm (infrared diodes)</td>
</tr>
<tr>
<td>LEDT frequency</td>
<td>continuous</td>
</tr>
<tr>
<td>Exit power</td>
<td>10mW (red diodes) and 30mW (infrared diodes)</td>
</tr>
<tr>
<td>LED size</td>
<td>0.2cm² (for both – red and infrared diodes)</td>
</tr>
<tr>
<td>Power density</td>
<td>0.05W/cm² (for red diodes) and 0.15W/cm² (for infrared diodes)</td>
</tr>
<tr>
<td>Irradiated energy</td>
<td>41.7 Joules on each point (0.3J for each red diode and 0.9J for each infrared diode)</td>
</tr>
<tr>
<td>Energy density</td>
<td>1.5 J/cm² on each point (for red diodes) and 4.5J/cm² on each point (for infrared diodes)</td>
</tr>
<tr>
<td>Treatment time</td>
<td>30 seconds on each point</td>
</tr>
<tr>
<td>Number of irradiation points per muscle</td>
<td>2</td>
</tr>
<tr>
<td>Total energy transmitted per muscle</td>
<td>83.4 Joules</td>
</tr>
<tr>
<td>Application manner</td>
<td>still on contact with skin at a 90-degree angle with light pressure</td>
</tr>
</tbody>
</table>
Comparison between the variation in the TBARS levels at the active LEDT and placebo LEDT situations (*p = 0.02).

DISCUSSION
In this preliminary study, we assessed the effect of LEDT applied before high-intensity exercise performed on cycle ergometer on the variation in the TBARS concentration. Despite having presented some limitations which will be approached later on, our work found positive results concerning LEDT as a prevention modality to oxidative stress induced by exercise. Such speculation is based on the increase in the TBARS levels in the situation at which the athletes received placebo LEDT application, contrasting with the balance observed in the situation at which the athletes received active LEDT application.

Positive results of the phototherapy on the oxidative stress have been reported in studies in which the LLLT was applied on the muscles of rats submitted to mechanical trauma(17,18), Fillipin et al.(17), using gallium arsenide laser (904nm, 45mW and 5J/cm²), found reduction of histological abnormalities as well as inflammatory response, besides increase in the collagen concentration in the group irradiated with LLLT. The authors also observed significant effects of LLLT in the reduction of oxidative stress, represented by the decrease in the TBARS levels compared to the group which did not receive laser. Moreover, increase in the enzymatic activity of the superoxide dismutase (SOD), an important antioxidant agent, was also observed.

Rizzi et al.(18) corroborate the previous evidence mentioned in a study using similar LLLT parameters on the mechanical trauma in muscles of rats. The authors observed considerable decrease of histological abnormalities, inflammatory response as well as TBARS levels, besides a blocking effect in the expression of the synthesis of nitric oxide (iNos) and in the activation of the nuclear factor Kb (NF-kB). The authors concluded that LLLT reduces the inflammatory response induced by trauma being able to block the negative effects of the release of ROSs.

Although the evidence in the literature has already mentioned the positive effect of phototherapy on oxidative stress prevention, it must be stressed that this study is a pioneer on assessing the LEDT effects previously applied to exercise in athletes with the aim to prevent lipid peroxidation induced by this kind of activity. Thus, although our findings cannot be compared with the literature, these can be considered a first step for studies on this new knowledge field.

Considering that this research was carried out as a preliminary study, some limitations should be mentioned, the first one regarding the sample size. The limited number of participants does not allow further statements on the results and makes it difficult to apply a more suitable statistical analysis. Additionally, we believe that athletes may not be the optimum population for the assessment of oxidative stress, since the systematic practice of physical exercises, especially of aerobic nature, increases the antioxidant capacity of an individual, and consequently, inhibits the installation of the phenomenon known as oxidative stress(26,27). Therefore, further studies involving untrained or sedentary subjects will probably promote more significant increase in the oxidative stress compared to our findings, as the ones observed by Chang et al.(28), who have also used TBARS as an assessment parameter.

The second limitation includes the exercise protocol used in the study. The Wingate test has a predominantly aerobic characteristic, being hence a test of maximum power and short time duration(28). In predominantly aerobic exercises, the increase in the oxygen supply to the mitochondria provides increase in the production of EROs(30). Although predominantly aerobic exercises are performed with limited oxygen participation, excessive production of ROSs can be also observed(31), probably by other mechanisms such as activation of xanthine oxidase, acidosis and oxidation of catecholamines(32). However, since the literature presents controversial results, we understand long-duration exercises are more suitable when the aim is to induce oxidative stress, especially in trained individuals.

Moreover, as the assessment of the oxidative stress in humans is a complex procedure, added to the fact that there is no biological marker considered gold standard, we emphasize the importance of using more than one biochemical analysis to determine oxidative stress(33). We suggest the observation of the antioxidant enzymes behavior – such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR) – which should be united with TBARS. Such observation seems appropriate since many researches believe that phototherapy increases the antioxidant activity through a photochemical process which accelerates the release of ROSs(17,34). Thus, the explanation for our findings may be related to the stimulating effect of phototherapy on the activity of antioxidant enzymes.

Finally, it is stated that despite the methodological limitations of the present study in proposing more concrete conclusions, our results demonstrate a protective effect of LEDT on the oxidative stress induced by exercise, besides being a pioneer in performing such verification in athletes. Further studies should be developed, since LEDT can become a new and non-pharmacological antioxidant agent as well as a prevention method to oxidative stress.

All authors have declared there is not any potential conflict of interests concerning this article.
REFERENCES