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ORIGINAL ARTICLE

Time response of increases in ATP and muscle resistance to fatigue after low-level laser (light) therapy (LLLT) in mice

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Abstract Recently, low-level laser (light) therapy has been used to increase muscle performance in intense exercises. However, there is a lack of understanding of the time response of muscles to light therapy. The first purpose of this study was to determine the time response for light-emitting diode therapy (LEDT)-mediated increase in adenosine triphosphate (ATP) in the soleus and gastrocnemius muscles in mice. Second purpose was to test whether LEDT can increase the resistance of muscles to fatigue during intense exercise. Fifty male Balb/c mice were randomly allocated into two equal groups: LEDT-ATP and LEDT-fatigue. Both groups were subdivided into five equal subgroups: LEDT-sham, LEDT-5 min, LEDT-3 h, LEDT-6 h, and LEDT-24 h. Each subgroup was analyzed for muscle ATP content or fatigue at specified time after LEDT. The fatigue test was performed by mice repeatedly climbing an inclined ladder bearing a load of 150 % of body weight until exhaustion. LEDT used a cluster of LEDs with 20 red (630± 10 nm, 25 mW) and 20 infrared (850±20 nm, 50 mW) delivering 80 mW/cm^2 for 90 s (7.2 J/cm^2) applied to legs, gluteus, and lower back muscles. LEDT-6 h was the subgroup with the highest ATP content in soleus and gastrocnemius compared to all subgroups ($P<0.001$). In addition, mice in LEDT-6 h group performed more repetitions in the fatigue test ($P<0.001$) compared to all subgroups: LEDT-sham and LEDT-5 min (~600 %), LEDT-3 h (~200 %), and LEDT-24 h (~300 %). A high correlation between the fatigue test repetitions and the ATP content in soleus ($r=0.84$) and gastrocnemius ($r=0.94$) muscles was observed. LEDT increased ATP content in muscles and fatigue resistance in mice with a peak at 6 h. Although the time response in mice and humans is not the same, athletes might consider applying LEDT at 6 h before competition.

Keywords Light-emitting diode therapy · Muscle ATP content · Photobiomodulation · Resistance to exercise fatigue · Time response

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Introduction

Low-level laser (light) therapy (LLLT) uses visible or near-infrared light to produce photobiomodulation in biological tissues which can either stimulate or inhibit biological responses depending on dose. Since 1967, this therapy has been used for several applications in medicine to treat disorders such as inflammation, pain, or accelerate healing [1–3]. Light therapy can be delivered by laser diodes, light-emitting diodes

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(LEDs), or other light sources with specific wavelengths ("colors") [1].

Several researchers have investigated the mechanism of how LLLT interacts with biological tissues to produce the photobiomodulation phenomenon [4–6]. One of first studies published reported the formation of "giant mitochondria" after the use of light therapy [7]. Several studies discovered molecules (called chromophores) inside cells that could absorb the light producing modulations in cell metabolism [4]. Among these molecules, cytochrome c oxidase (Cox) which is the complex IV of the mitochondrial electron transport chain has received special attention [4, 8–11].

Recently, Hayworth et al. [12] used an array of LED (660 nm) to increase Cox activity after 24 h after light therapy when applied to rat muscles without contact. These authors reported differences in Cox activity depending on the type of muscle fibers.

Increased Cox activity is supposed to be responsible for stimulating the synthesis of adenosine triphosphate (ATP) [4]. Karu [4] reported several years ago that light therapy effects on biological tissues can be classified as either primary (light-tissue interactions) or secondary mechanisms (photobiomodulation). One of the most frequently observed effects of photobiomodulation is the increased ATP synthesis in cultured cells [4, 13, 14].

Increased Cox activity resulting in more ATP synthesis has been the principle explanation in studies that have used LLLT to increase muscle performance before (muscular pre-conditioning) or after (muscle recovery) different types of exercise [15–17]. Combined with other metabolic products as lactate and accumulation of adenosine diphosphate (ADP), ATP synthesis is important for optimum muscle performance since reduced levels of ATP in muscles have been held responsible for lowered resistance of muscles to fatigue and decreased performance in many types of exercise [18].

The scientific literature reports several strategies and therapies to increase muscle performance in different sports. However, several drugs have been prohibited from being used for this purpose as they are considered "doping" [19]. With this perspective in mind, over the last decade, some researchers have used light therapy to promote muscular pre-conditioning and to increase performance in intense exercise [15–17]. These reports have found good effects with this regimen of light therapy in muscular pre-conditioning, preventing muscle damage, and increasing the number of repetitions in fatigue tests. However, the majority of these results have only reported a small improvement compared to control groups, mainly measuring the number of repetitions in fatigue tests [20–22]. There may be a lack of understanding of the time response of muscles to light therapy and uncertainty about the best time to apply the light for optimal fatigue-muscle resistance in exercise until exhaustion.

The present study aimed to investigate (a) if light-emitting diode therapy (LEDT) can increase muscle ATP synthesis in vivo, (b) the possible time response for ATP synthesis in gastrocnemius and soleus muscles in mice mediated by LEDT, (c) the possible time response after LEDT for increased muscle performance in fatigue test, and (d) correlations between ATP contents in soleus and gastrocnemius muscles with the fatigue test. This study suggests that LEDT may be a new strategy for improved muscle performance as well as indicates which time is better to use light therapy in muscular pre-conditioning.

Materials and methods

Animals

This study was performed with 8-week-old male Balb/c mice, weighing on average 20.38±1.10 g, housed at five mice per cage, and kept on a 12-h light 12-h dark cycle. All 50 animals were provided by Charles River Inc and were treated with water and fed ad libitum at Animal Facility of the Massachusetts General Hospital. All procedures were approved by the IACUC of the Massachusetts General Hospital and met the guidelines of the National Institutes of Health.

Experimental groups

Fifty mice were randomly allocated into two equal groups: LEDT-ATP and LEDT-fatigue. ATP contents in gastrocnemius and soleus muscles were evaluated in animals allocated into LEDT-ATP group. Fatigue-muscle resistance was analyzed in animals allocated into LEDT-fatigue group. Each one of these groups was subdivided into five equal subgroups (n=5) with random allocation of the mice (Fig. 1):

1. LEDT-ATP subgroups

   - LEDT-sham: animals were treated with LEDT placebo (LEDT device in placebo mode) over both legs, gluteus, and lower back muscles immediately before (5 min) surgery procedures and sacrifice.
   - LEDT-5 min: animals were treated with real LEDT over both legs, gluteus, and lower back muscles immediately before (5 min) surgery procedures and sacrifice.
   - LEDT-3 h: animals were treated with real LEDT over both legs, gluteus, and lower back muscles 3 h before surgery procedures and sacrifice.
   - LEDT-6 h: animals were treated with real LEDT over both legs, gluteus, and lower back muscles 6 h before surgery procedures and sacrifice.

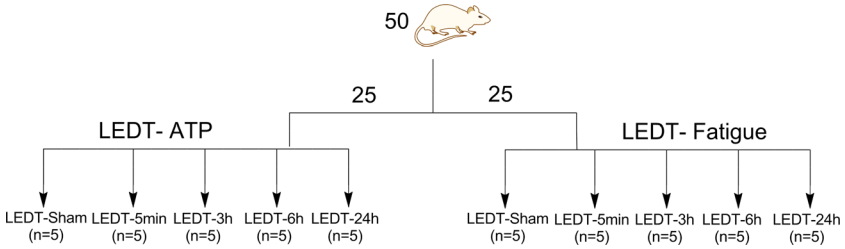

Fig. 1 Randomization and groups. Fifty male Balb/c mice were first allocated into two equal groups: LEDT-ATP and LEDT-fatigue. Next, both groups were subdivided into five equal groups: LEDT-sham, LEDT-5 min, LEDT-3 h, LEDT-6 h, and LEDT-24 h. *LEDT* light-emitting diode therapy, *ATP* adenosine triphosphate

- LEDT-24 h: animals were treated with real LEDT over both legs, gluteus, and lower back muscles 24 h before surgery procedures and sacrifice.

2. LEDT-fatigue subgroups

- LEDT-sham: animals were treated with LEDT placebo (LEDT device in placebo mode) over both legs, gluteus, and lower back muscles immediately before (5 min) fatigue test on inclined ladder.
- LEDT-5 min: animals were treated with real LEDT over both legs, gluteus, and lower back muscles immediately before (5 min) fatigue test on inclined ladder.
- LEDT-3 h: animals were treated with real LEDT over both legs, gluteus, and lower back muscles 3 h before fatigue test on inclined ladder.
- LEDT-6 h: animals were treated with real LEDT over both legs, gluteus, and lower back muscles 6 h before fatigue test on inclined ladder.
- LEDT-24 h: animals were treated with real LEDT over both legs, gluteus, and lower back muscles 24 h before fatigue test on inclined ladder.

Procedures

Familiarization with climbing ladder

An inclined ladder (80°) with 100×9 cm (length and width, respectively) with bars spaced at 0.5-cm intervals was used in this study as reported in a previous study [23]. However, the maximum distance available to climb was set at 70 cm in order to avoid possible contact between the load and the floor [24] (Fig. 2). The familiarization procedure was set as 4 sets of 10 climbs on ladder (repetitions) with rest times of 2 min between sets. No load was attached to the mouse tail during this procedure as reported in the previous study [24]. Animals allocated into LEDT-fatigue subgroups were familiarized to climb the ladder 2 days before the start of the fatigue test.

Light-emitting diode therapy

This study used a non-commercial cluster of 40 LEDs of 76 mm: 20 red LEDs (630±10 nm) and 20 infrared LEDs (850±20 nm). LEDT parameters presented in Table 1 are the same used in the previous study [24]. The optical power

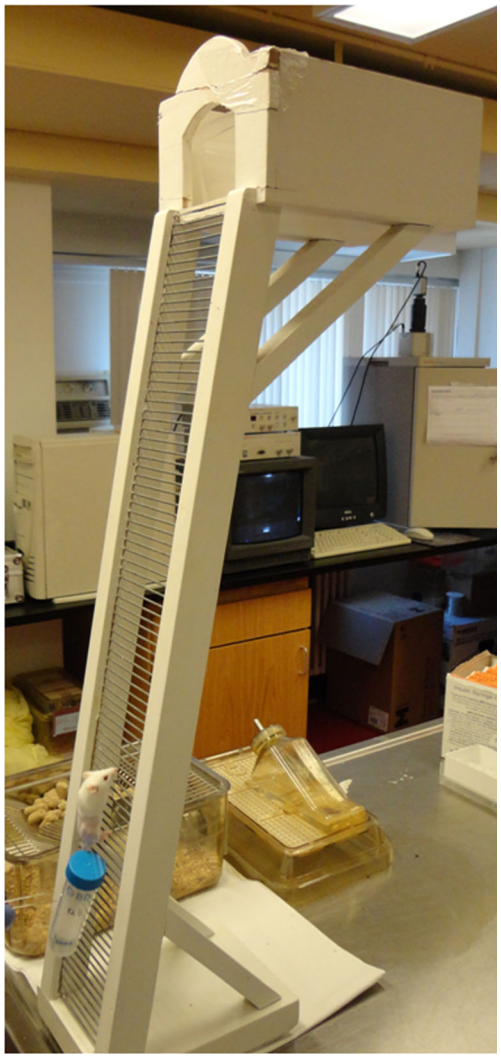

Fig. 2 Ladder. Inclined ladder (80°) with 100×9 cm (length and width, respectively) used for the fatigue test. Falcon tube filled with water and attached to mouse tail

Table 1 Optical parameters of light-emitting diode therapy (LEDT). LEDT-sham group received a placebo therapy (device switched off) with the same time of treatment

Number of LEDs (cluster): 40 (20 infrared-IR and 20 red-RED)
Wavelength: 850±20 nm (IR) and 630±10 nm (RED)
Pulse frequency: continuous
Optical output of each LED: 50 mW (IR) and 25 mW (RED)
Optical output (cluster): 1,000 mW (IR) and 500 mW (RED)
LED cluster size: 45 cm^2
Power density (at skin surface): 80 mW/cm^2
Treatment time: 90 s
Energy density applied (at skin surface): 7.2 J/cm^2
Application mode: without contact
Distance from mice or power meter: 45 mm

(power density) and energy density of LED cluster was measured with an optical and energy meter PM100D Thorlabs® and sensor S142C (area of 1.13 cm^2) at a distance of 45 mm as described previously [24]. Mice were shaved and fixed on a plastic plate using adhesive tapes without anesthesia. Afterwards in accordance with experimental subgroups, all animals were treated with LEDT over both legs, gluteus, and lower back muscles at a distance of 45 mm (without contact) [24] (Fig. 3). Irradiation lasted 90 s per session with fixed parameters as described in Table 1. LEDT placebo had no energy (0 J) and power (0 mW) applied over these muscles. The temperature on the mice skin was monitored before LEDT irradiation and after 5 min using a precise thermometer. There was no change observed on temperature.

Anesthesia, surgical, and sacrifice procedures

All mice of LEDT-ATP group were subjected to anesthesia, surgery, and sacrifice procedures at the specified time after LEDT for each subgroup. Animals of LEDT-fatigue subgroups after finishing fatigue test were anesthetized and sacrificed immediately.

Anesthesia Mice were anesthetized using ketamine and xylazine at a proportion of 80 mg/kg of ketamine and 12 mg/kg of xylazine.

Surgery After the anesthesia procedure, the gastrocnemius and soleus muscles were excised bilaterally, separated surgically, and immediately frozen in liquid nitrogen. Next, both muscles were stored at −80 °C until analysis of ATP performed exactly 7 days after the surgery.

Sacrifice Animals were sacrificed under anesthesia by cervical dislocation at same period of day (afternoon).

It is important to remark the order of the procedures conducted in this study. The first procedure was irradiating all mice of LEDT-ATP subgroups (except LEDT-Sham) with LEDT. During 7 days between sacrifice of these animals and ATP analysis, the fatigue test with mice of the LEDT-fatigue subgroups was performed.

Outcomes

Muscular ATP content

The gastrocnemius and soleus muscles from one leg of each animal were used for this analysis. Muscle samples were thawed in ice for 5 min and homogenized at a proportion of 3–4 mg of tissue to 500 μl of 10 % perchloric acid ($HClO_4$) following procedures previously published [24, 25]. Afterwards, an aliquot of 10 μl of the muscle homogenate plus 40 μl of CellTiter Glo Luminescent Cell Viability Assay kit (Promega), totaling 50 μl, was placed in a 96-well microplate (Costar™ 96-Well White Clear-Bottom Plates). Luminescence signals were measured in a SpectraMax M5 Multi-Mode Microplate Reader (Molecular Devices, Sunnyvale,

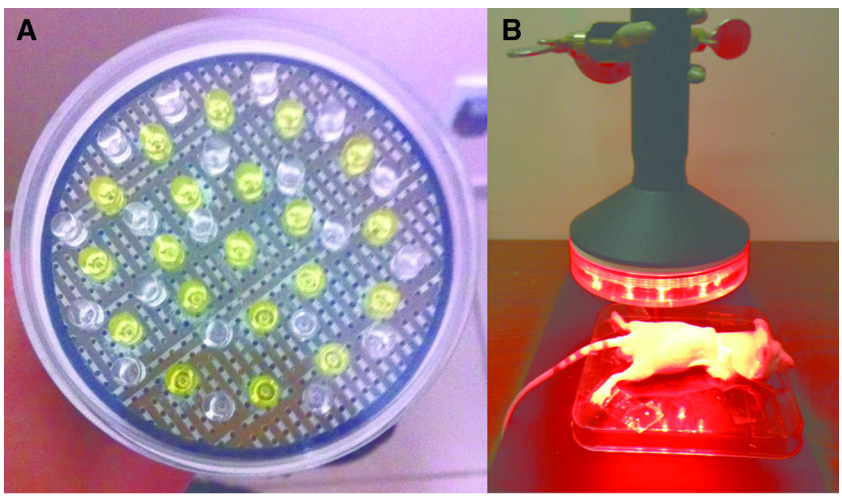

Fig. 3 LEDT. **a** Internal distribution of light-emitting diodes (LEDs) in the cluster. White LEDs emit red (630±10 nm) and yellow LEDs emit infrared (850±20 nm). **b** Positioning of the mice to receive LED therapy (LEDT) over legs, gluteus, and lower back muscles without contact

CA) with integration time of 5 s to increase low signals [25]. A standard curve was prepared using ATP standard (Sigma) according to manufacturer's guideline and then ATP concentration was calculated in nanomole (nmol) per milligram (mg) of protein. An aliquot of muscle homogenate was used to quantify the total protein by QuantiPro™ BCA Assay kit (Sigma-Aldrich) following manufacturer's guidelines.

Fatigue test

This test was performed 48 h after the familiarization procedure with a load corresponding to 150 % of the mice body weight. All animals allocated into LEDT-fatigue subgroups were weighed on a precise scale and then the target load was calculated. A Falcon tube (50 ml) was filled with specific milliliters of water until the total matched the target load in grams [24]. Next, this tube was attached to the mouse tail using adhesive tape (Fig. 2). Mice performed this test exactly 5 min, or 3 h, or 6 h, or 24 h after LEDT procedure in accordance with LEDT-fatigue subgroups. Slight pressures with tweezers were applied to the mouse tail if the animal stopped climbing. The test stopped when mice were not able to climb or lost their grip and slid on the ladder due to failure of concentric muscle contraction after. The number of climbs on the ladder (repetitions) of each mouse was quantified during this test. The room temperature was monitored and kept on 22–25 °C.

Pearson product-moment correlation coefficient (Pearson's r)

Correlations were calculated between ATP contents in soleus muscle and number of repetitions in the fatigue test, as well as ATP contents in gastrocnemius muscle and fatigue test. The *r* values were interpreted as recommended previously [26]: 0.00–0.19=none to slight, 0.20–0.39=low, 0.40–0.69=modest, 0.70–0.89=high, and 0.90–1.00=very high.

Sample size calculation

The sample size was calculated based on the necessary number of animals to obtain significant differences among the groups regarding ATP content in soleus and gastrocnemius and the number of repetitions in fatigue test. The statistical power of 80 % and the effect size (greater than 0.75), and alpha (α) of 5 % were found to be satisfactory.

Statistical analysis

Shapiro-Wilk's *W* test verified the normality of the data distribution. ATP contents in soleus and gastrocnemius muscles among all groups were compared using one-way analysis of variance (one-way ANOVA) with Tukey HSD post hoc test. Pearson product-moment correlation coefficient (Pearson's *r*) was conducted between fatigue test and ATP contents in soleus and gastrocnemius muscles. Significance was set at $P<0.05$.

Results

ATP content in soleus muscle

ATP content in soleus was modulated significantly by LEDT and presented a time response for this photobiomodulation. The group LEDT-6 h (85.80±16.41 nmol/mg protein) had the highest ATP content in soleus compared to LEDT-sham (27.48±2.28 nmol/mg protein; $P<0.001$), LEDT-5 min (36.62±12.36 nmol/mg protein; $P<0.001$), LEDT-3 h (57.92±7.40 nmol/mg protein; $P=0.011$), and LEDT-24 h (45.54±13.84 nmol/mg protein; $P<0.001$). The second group with more ATP content was LEDT-3 h when compared to LEDT-sham ($P=0.005$), but LEDT-3 h had no statistical difference compared to LEDT-5 min ($P=0.070$) and LEDT-24 h ($P=0.491$). LEDT-24 h had no difference in ATP content compared to LEDT-sham ($P=0.158$) and LEDT-5 min ($P=0.761$). Finally, LEDT-5 min had no difference in ATP content compared to LEDT-sham ($P=0.745$) (Fig. 4a).

ATP contents in gastrocnemius

Similarly to ATP content in soleus muscles, the group LEDT-6 h (142.30±11.13 nmol/mg protein) had also the highest ATP content in gastrocnemius muscle compared to LEDT-sham (18.71±4.27 nmol/mg protein; $P<0.001$), LEDT-5 min (23.30±3.14 nmol/mg protein; $P<0.001$), LEDT-3 h (87.67±15.66 nmol/mg protein; $P<0.001$), and LEDT-24 h (39.72±10.76 nmol/mg protein; $P<0.001$). LEDT-3 h was the second group with highest ATP content in gastrocnemius muscle compared to LEDT-sham ($P<0.001$), LEDT-5 min ($P<0.001$), and LEDT-24 h ($P<0.001$). In addition, LEDT-24 h had higher ATP content compared to LEDT-sham ($P=0.028$) but without statistical difference compared to LEDT-5 min ($P=0.117$). Finally, LEDT-5 min had no difference in ATP content compared to LEDT-sham ($P=0.950$) (Fig. 4b).

Fatigue test

LEDT-6 h was the best group among all LEDT-fatigue subgroups, performing 67.40 (±3.05) repetitions with significant differences compared to LEDT-sham (11.86±0.89 repetitions; $P<0.001$), LEDT-5 min (13.90±0.82 repetitions; $P<0.001$), LEDT-3 h (31.04±3.42 repetitions; $P<0.001$), and LEDT-24 h (23.60±3.02 repetitions; $P<0.001$). LEDT-3 h was the second best group, performing more repetitions compared to LEDT-sham ($P<0.001$), LEDT-5 min ($P<0.001$), and LEDT-

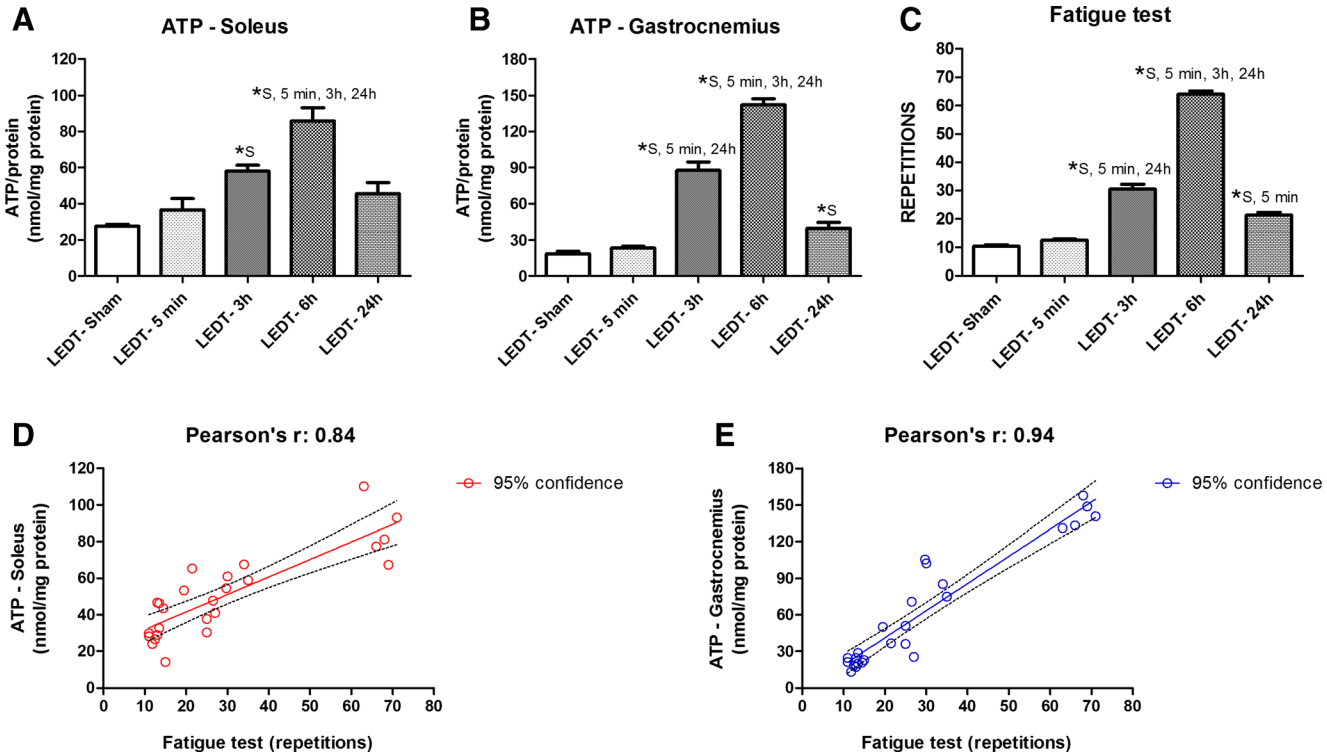

Fig. 4 ATP, fatigue test, and correlations (n=5 animals per subgroup). **a** ATP contents in soleus muscle for LEDT-ATP subgroups: LEDT-sham, LEDT-5 min, LEDT-3 h, LEDT-6 h, and LEDT-24 h. **b** ATP contents in gastrocnemius muscle for LEDT-ATP subgroups: LEDT-sham, LEDT-5 min, LEDT-3 h, LEDT-6 h, and LEDT-24 h. **c** Fatigue test for LEDT-fatigue subgroups: LEDT-sham, LEDT-5 min, LEDT-3 h, LEDT-6 h, and LEDT-24 h. **d** Pearson's r correlation between ATP contents in soleus muscle and fatigue test. **e** Pearson's r correlation between ATP contents in gastrocnemius and fatigue test. *LEDT* light-emitting diode therapy, *ATP* adenosine triphosphate, *S* LEDT-sham, *5 min* LEDT-5 min, *3 h* LEDT-3 h; *24 h* LEDT-24 h; *P<0.05 (statistical significance)

24 h (P=0.001). LEDT-24 h performed more repetitions compared to LEDT-sham (P<0.001) and LEDT-5 min (P<0.001). Finally, LEDT-5 min presented no statistical difference compared to LEDT-sham (P=0.700) (Fig. 4c).

Sample size

Statistical power and effect size regarding the ATP content in soleus and gastrocnemius muscles among all groups were calculated in order to ensure the minimal power of 80 %, alpha (α) of 5 %, and large effect size (greater than 0.75). We used the mean of ATP content of the each LEDT-ATP subgroup and the highest value of standard deviation among all these subgroups. For ATP content in soleus, our results demonstrate a difference between groups with a statistical power of 87 %, effect size of 1.23 (very large effect size), and total sample size of 15, i.e., three animals per group (five groups). For ATP content in gastrocnemius, our results demonstrate a difference between groups with a statistical power of 99 %, effect size of 2.99 (huge effect size) and total sample size of 10, i.e., two animals per group (five groups). For fatigue test, we used the same criteria for ATP content in muscles: minimal power of 80 %, alpha (α) of 5 %, and large effect size (upper to 0.75). Using the mean of repetitions of each LEDT-fatigue subgroup and the highest value of standard deviation among all these subgroups, our results demonstrate a difference between groups with a statistical power of 100 %, effect size of 5.88 (huge effect size), and total sample size of 10, i.e., two animals per group (five groups). All these calculations demonstrate that sample size, power, and effect size of this study were adequate for ATP content in soleus and gastrocnemius, and for fatigue test, supporting the conclusion regarding the time response of muscles to LEDT observed in this current study.

Pearson product-moment correlation coefficient (Pearson's r)

ATP contents in soleus and gastrocnemius muscles presented a high correlation (r=0.84) and very high correlation (r=0.94) with the number of repetitions performed in the fatigue test (P<0.001; P<0.001), respectively (Fig. 4d, e).

Discussion

Our study found strong positive effects of LEDT for increasing muscle ATP synthesis in vivo, as well as establishing the time response for this photobiomodulation phenomenon. We found also similar results for the LEDT-mediated increase in

fatigue-muscle resistance in the exercise test performed on an inclined ladder. In addition, contents of muscle ATP and fatigue-muscle resistance were highly correlated. To our knowledge, this is the first study reporting the time response for the effects of light therapy on muscle ATP synthesis and fatigue-muscle resistance in vivo.

Since cytochrome c oxidase (Cox) has been reported as the principle chromophore in cells [4, 8–11], changes in mitochondrial metabolism, oxidative stress, and increased ATP synthesis have been considered important mechanisms in LLLT [4]. Therefore, this study used light therapy to modulate ATP synthesis as has already been done previously in vitro [4, 13, 14] but explored what was the best time to apply the light before the exercise.

Previous studies have used light therapy for muscular preconditioning by delivering the light therapy over the target muscles 5 min before fatigue tests in vivo [27, 28] or in clinical trials [20–22]. These studies reported an increased number of repetitions and consequently a better fatigue-muscle resistance. Our results for fatigue-muscle resistance on the ladder for LEDT-3 h, LEDT-6 h, and LEDT-24 h groups confirmed these previous results, except for LEDT-5 min. The number of repetitions was greatly increased in LEDT-6 h (~600 %), LEDT-3 h (~300 %), and LEDT-24 h (~200 %) compared to LEDT-sham and LEDT-5 min, thus establishing a well-defined time response for LEDT to increase fatigue-muscle resistance. Although LEDT-5 min slightly increased the number of climbs on ladder (around 20 %) compared to LEDT-sham, this increase was really small and for this reason had no significance. A study with a larger number of animals would be necessary to provide the statistical power to prove this difference. Possibly, the use of a *t* test between LEDT-sham and LEDT-5 min groups could show a statistically significant difference, such as has been reported in previous studies involving LLLT and muscular pre-conditioning [20–22].

Hayworth et al. [12] reported modulations in Cox activity 24 h after the use of light therapy on the temporalis muscles in rats, but these modulations were dependent on the type of metabolism in muscle fibers that in turn suggested differences in energy synthesis (ATP). For this reason, our study assessed the effect of LEDT on ATP synthesis in muscles with either a predominance of aerobic metabolism (soleus) and mixed aerobic and glycolytic metabolism (gastrocnemius) [29]. Our results clearly show an increased ATP synthesis in both types of muscles after LEDT, both responses showing a well-defined time response. Previous studies already reported increased ATP synthesis in cells after light therapy [4, 13, 14]. In our study, we show that increases in ATP occur in muscle tissue occurring over a wide time range (5 min to 24 h) showing that secondary reactions [4] occur over time in vivo.

Increased ATP content in muscle tissue suggests more energy available for all metabolic processes, including muscle contraction [18, 24]. Corroborating this statement, our results for muscular ATP content and fatigue-muscle resistance were highly correlated, reinforcing the importance of a good energy supply to achieve the best performance in exercise [18]. In the context of energy metabolism, previous studies already reported possible effects of light therapy on resynthesis of creatine-phosphate (Cr-P) using ATP produced in mitochondria, as well as the consumption of lactate produced by anaerobic metabolism during fatigue or strength exercise [15, 30, 31]. Therefore, we believe that these mechanisms could provide better energy restore/supply during the fatiguing exercise.

Looking more deeply into our results, we observed that although the increased ATP content in soleus muscle showed how mitochondrial metabolism was stimulated by light therapy, the gastrocnemius muscle showed the best correlation (Pearson's $r=0.94$) with the fatigue test. The gastrocnemius muscle has a mixed metabolism (oxidative and glycolytic) [29] and perhaps light therapy could modulate both of these different metabolic pathways. In summary, our results suggest that both glycolytic and oxidative metabolisms were stimulated by light therapy, considering that mitochondria need acetyl coenzyme A (acetyl-CoA) coming from glycolysis and/or from β-oxidation to perform ATP synthesis.

The power density (irradiance) and dose (fluence) of the light therapy used in this current study were based on the possible biphasic dose response reported previously [5, 1]. However, we used red and near-infrared light (two wavelengths) delivered at the same time based on specific absorptions of the chromophores in the cells to absorb these lights [4, 8–11]. It is possible that taking advantage of the double absorption bands (red and near-infrared together) could optimize the effects of photobiomodulation to promote ATP synthesis and fatigue-muscle resistance. Moreover, light therapy was delivered without contact as reported previously [12, 24], covering all target muscles (gastrocnemius, soleus, gluteus, and lower back muscles) and made it possible to stimulate entire muscle groups simultaneously as reported previously [31]. Finally, as the light irradiation was performed without contact, possibly reflection of the light on the animal surface (mainly on the curved areas) would have occurred in our study. This phenomenon could promote loss of photons penetrating through the skin and reaching the muscles. However, light-tissue interaction is strongly dependent on the power of the light (Beer-Lambert law) and the chromophores that absorb this light [4, 8–11]. For these reasons, light irradiation with large LED array was without contact (instead of point by point using a small beam area) in order to cover entire area of all muscles at the same time.

Conclusion

This is the first study reporting a well-defined time response for improving both muscle ATP content and also resistance to

fatigue by mixed red and near-infrared light therapy applied over skeletal muscles.

Our data presented in this study could be used in future studies that aim to determine whether a similar time response of muscles after LEDT applies in humans with an adjusted light dose for humans. It may be possible to use light therapy to enhance performance in athletics and high-performance sports, as well as in a myriad of different medical or health science applications.

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